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THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXXII—No. 3
Issued May 1, 1925

BALTIMORE, U. S. A.
1925

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 72 MAY 1, 1925 No. 3

VOLUNTARY RELAXATION OF THE ESOPHAGUS

EDMUND JACOBSON

From the Hull Physiological Laboratory of the University of Chicago

Received for publication January 30, 1925

Whether the esophagus is as far removed from voluntary influence as is commonly believed became open to question during studies of spasm. Individuals able to change their heart rate generally have been regarded as rare freaks or anomalies and have not been systematically studied. No instance of voluntary influence, so far as I know, has been reported about the esophagus, which receives its motor supply in man from the vagus. Accordingly it has seemed of interest to enquire whether the organ can be voluntarily relaxed, the more especially since this may throw new light upon the relations of the cerebrospinal and autonomic systems.

The opportunity to make the study arose through a method which has been recently described (1). After many hours of instruction and practice, devoted not alone to the skeletal muscles of the limbs and trunk, but also to those of the eyes and speech, the individual learns to attain a general flaccidity of extreme degree. Various investigations are under way and further effects of this flaccidity will soon be reported. With this method, it became possible to study voluntary influence upon the esophagus during both normal and abnormal conditions.

Three persons were used for study. Subject 1 was a university student of nineteen, who since June, 1920, apparently had suffered most of the time every day with painful spasm. On one occasion, during May, 1923, to aid the experiment, he had remained up most of the previous night and had taken vigorous exercise,—a combination which had generally in his experience been followed by severe distress. Spasm was observed under the fluoroscope for an hour, upon swallowing of barium paste at intervals. Then after 20 minutes of voluntary relaxation, barium was again given. This time no spasm was shown on the film or under the fluoroscope. Similar observations were made in August. Persistent spasm was found (fig. 1), then the subject relaxed for about 20 minutes, whereupon the

barium paste was given again, but this time was seen to pass normally through the esophagus, which evidently was no longer spastic.

Control tests show that the normally acting esophagus empties directly or within a minute or two, if the paste used is not too dry. The paste is not held persistently, as in spasm, over periods of 5 minutes or more with partial stasis. It is of course necessary to distinguish between the barium persisting as a thick mass in the esophagus and merely as a thin coating adhering to the wall. The latter is not to be taken as evidence of spasm.

The following table of control tests was made with six males, assumed to be fairly normal. Paste consisted of barium sulphate, grams 220, chocolate malted milk, grams 16, water 30 cc. The last column indicates the time after swallowing for a large spoonful ($\frac{1}{8}$ of the above mixture) to leave the cardia.

SUBJECT	AGE	WEIGHT	HEIGHT	TIME
1	49	215	5 feet $8\frac{3}{4}$ inches	45 seconds
2	33	125	5 feet 7 inches	1 minute 50 seconds
3	24	133	5 feet 8 inches	1 minute
4	62	130	5 feet 4 inches	2 minutes
5	60	175	5 feet 10 inches	1 minute 8 seconds
6	36	142	5 feet 10 inches	1 minute 3 seconds

Subject 2 was a woman of 49, who spoke of "choking sensations" all day long for the past six weeks, most marked after meals. Since the age of 31, she had been subject to epileptic spells. Under the fluoroscope, spasm was severe. A spoonful of paste failed to leave the esophagus completely in 25 minutes (see fig. 2). Then she was ordered to relax so far as possible while continuing to stand. She had no previous training, but her head and limbs drooped unusually well for an unpracticed person. After 20 minutes more, the esophagus was empty. At the same time the "choking sensations" diminished. No hint of this result had previously been given. A month later, after the subject had been taught to be habitually relaxed, there was no evidence of spasm under the x-ray.

Subject 3 was a woman of 39. (A film of her esophagus will be found as fig. 1 in reference 2 at the end of this article.) For the past two days, she had complained of almost continual distress as from a "lump" or "ball" in the throat. At 1:40 p.m. she took 2 teaspoonsful of the barium mixture. Persistent spasm apparently involved the entire esophagus. At 1:53 more barium was given, and part of this remained for 25 minutes. The pylorus opened after 15 minutes, while the cardiac portion of the stomach also appeared spastic. At 2:35 she began to relax and 3:15 reported that she was free from the feeling as "from a stiff tube." At this time spasm no longer appeared under the fluoroscope, excepting a little above the cardia. The organ now emptied in 3 to 4 minutes with the same paste as previously used.

It was suggested by Professor Carlson that these observations be confirmed with kymograph tracings. A rubber bag about 3.8 cm. long was swallowed, attached to a fine rubber tube connected with a manometer. A thread 22.5 cm. long was attached to the proximal end of the bag and to a front tooth. The bag thus lay in about the middle portion of the esophagus. Upon inflation a record of contraction of the esophagus could be obtained. The subject lay upon a couch during the period of observation, making no bodily movement, for this would confuse the tracing. An early source of error arose from slight leaks of the air system. This was elimi-

Fig. 1

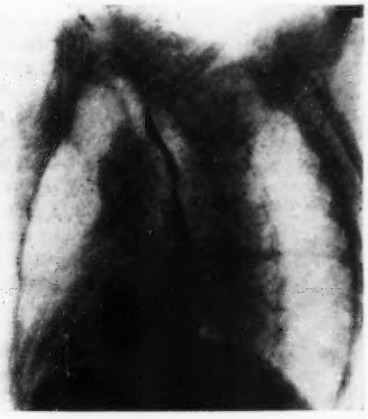

Fig. 2

Fig. 1. Subject 1. Spasm of the esophagus, confirmed under the fluoroscope. A film alone may not establish the presence of spasm, since it is necessary to show that the contraction is persistent. Later, when another portion of barium was given after about 20 minutes of general relaxation, the esophagus appeared wide and relaxed under the fluoroscope, emptying in normal manner practically at once.

Fig. 2. Subject 2. Spasm of the esophagus. Barium persistent for 25 minutes. After a period of general relaxation, the esophagus appeared relaxed under the fluoroscope and emptied in normal time when barium was again given.

nated by sealing the points of attachment of the hose to the glass tubing with collodion after they had been tied with surgical knots. It is necessary to test for a slight leak after every period of experimentation. The balloon is inflated, after being removed from the esophagus, and a tracing is made over a period of two hours, when there should be practically no fall.

Many sessions of practice proved necessary before subject 1 learned to relax during the additional distress from an inflated balloon in the esophagus. Control records were made in each instance to establish that the

contraction or spasm persisted before the signal to relax was given. A sample of the later records is shown in figure 3. It will be seen that the x-ray observations are confirmed.

Study of the records reveals various items of interest. The esophagus responds in its contractions to divers stimuli or disturbances, such as noises, the entrance of a visitor, flies alighting on the skin, or even thought-processes. When the disturbance awoke fear, the contraction was increased. Marked spasm seemed identical with fear to this subject. As the esophagus relaxed, the distress vanished correspondingly. The subject readily gained the ability to judge when his esophagus was tense. Comparison of the subjective reports with the tracings showed noteworthy correspondence. (This harmonizes with Carlson's observation that his subjects were able to judge the presence of hunger contractions (3).) The attempt to relax was sometimes successful in some measure practically at once, as shown by an abrupt fall of the curve; but more often the fall was slow and progressive. Sometimes the instruction to relax is followed by an unsuccessful attempt: the subject may fail to relax his skeletal muscles to a marked degree, as seen by gross observation, and may report his failure later. It may be necessary to give instructions to relax repeatedly and even to point out to the subject what muscles appear tense. When the esophagus once becomes well relaxed the subject may not by an effort make it tense again. We did not try to find out whether such ability could be cultivated with practice. After practice at relaxation has made the matter habitual, a certain degree of relaxation may take place *nolens volens* when the individual takes his accustomed position of rest, even if the instruction not to relax be given. As relaxation became habitual and distress less frequent, it became increasing difficult to excite spasm for experimental purposes by inflation of the balloon. When the subject seemed most relaxed, no swallowing took place even for periods of half an hour. This contrasts with the unrelaxed normal esophagus, where repeated swallowing is characteristic (4). This subject generally swallowed preliminary to carrying out the instruction to relax; therefore in later studies he was directed to swallow before the instruction to relax was given.

Tracings were made with subject 2 during the period of severe spasm. First a control test was made for half an hour to see whether the spasm disappeared spontaneously because of the supine position alone. After it had persisted, the instruction to relax was given. As shown in figure 4, there was a remarkable change in the contractions when the subject relaxed. This individual reported that during general relaxation, the "choking sensations" disappeared. No previous intimation to this effect had been made to her. As she practiced relaxation from day to day during the following month, marked spasm seemed to become more difficult to evoke upon inflation of the balloon, corresponding to the x-ray findings. Records (fig. 5) made after the spasm was no longer in evidence suggest that a progressive decline in contraction may follow the instruction to relax, which quite resembles the action of calcium chloride, atropin and other depressant chemicals on the esophagus, as shown in the tracings of Danielopolu and associates. These writers used small doses, and the effects apparently were shorter in duration than ours. There is similar

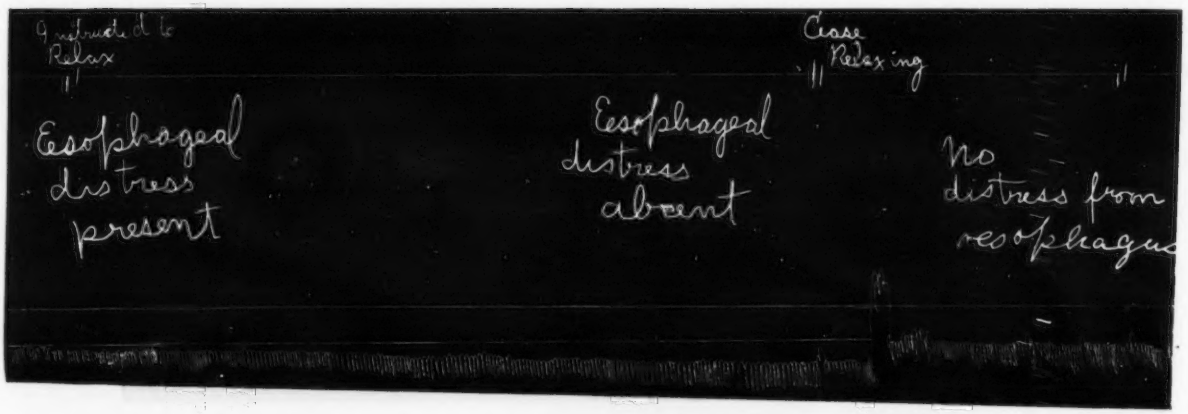

Fig. 3. Subject 1. Progressive relaxation of the esophagus is shown by the general fall of the curve measured from the base line. The swallowing reflex is absent here. Up and down strokes represent respiration. The total time during record shown is about 25 minutes.

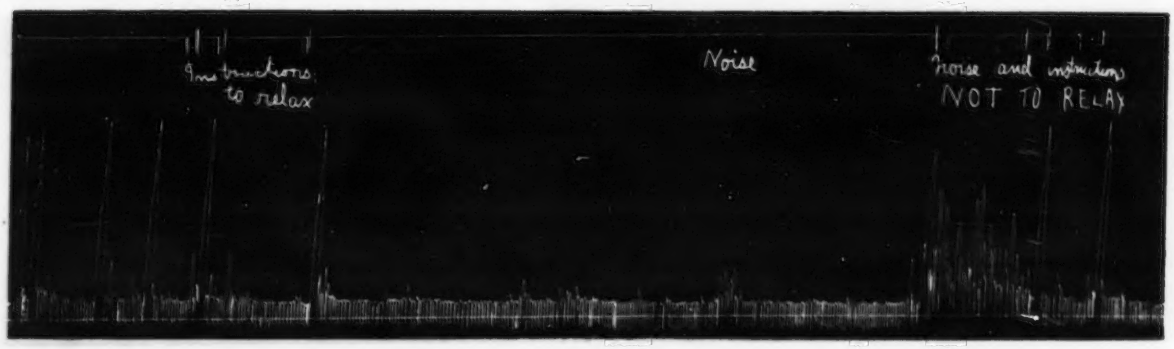

Fig. 4. Subject 2. Relaxation of the esophagus is shown by the fall of the curve corresponding with instructions.

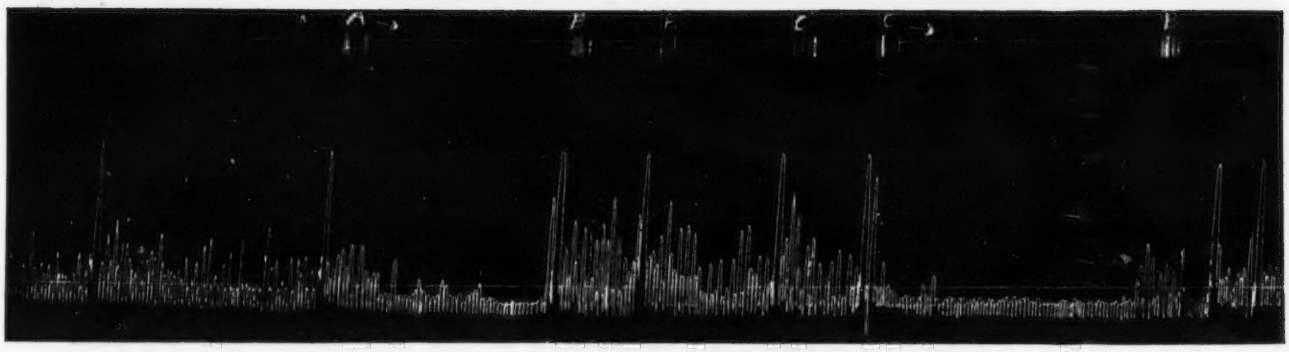

Fig. 5. Subject 2. This curve during voluntary relaxation closely resembles the records obtained by Danielopulu and associates for the effects of calcium chloride and other depressants. Breaks in upper line indicate *A*, signal to relax; *B*, to cease relaxing; *C*, prolonged instructions to relax.

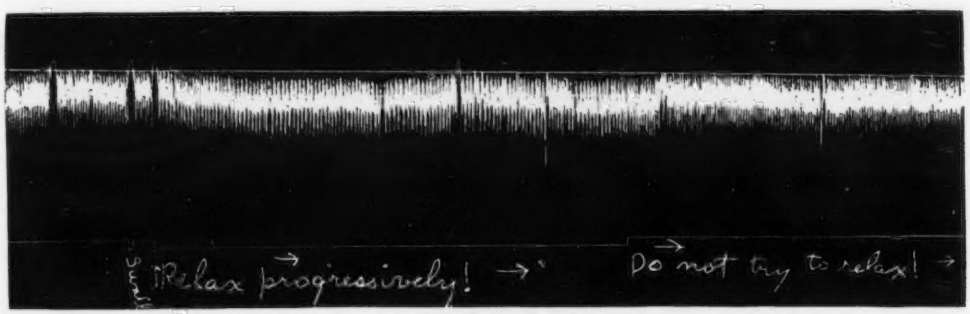

Fig. 6. Subject 1. Balloon just above cardia. This illustrates a slow progressive relaxation of the smooth muscle.

diminution of swallowing and of height of respiratory contraction as well as a general decline of the curve as compared with the base line.

Fluroscopic studies had shown that the lowermost portion of the esophagus acted like the upper portions during relaxation. To test this with subject 1, the balloon was introduced with a length of thread about 36 cm. A greater distance would have had the balloon in the cardia, as shown by the change in the tracing (5). Voluntary relaxation in this region of smooth muscle alone is illustrated in figures 6 and 7.

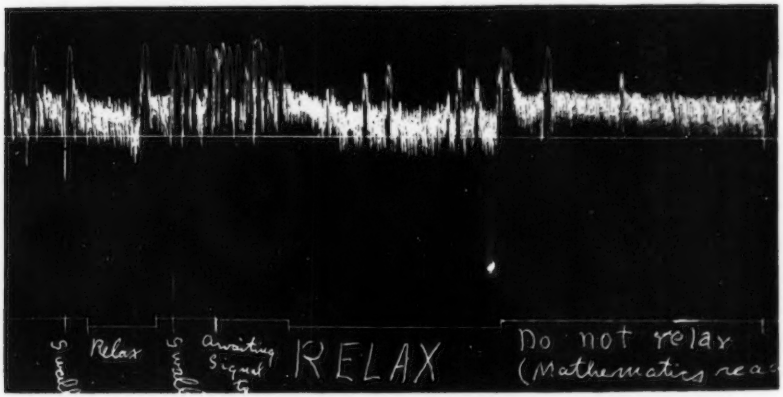

Fig. 7. Subject 1. Balloon just above cardia. The record illustrates almost immediate relaxation of the smooth muscle following the instruction to relax.

RÉSUMÉ AND CONCLUSIONS

1. X-ray and kymograph studies were made of the esophagus in individuals who had been trained to relax their skeletal muscles. It was found that the esophagus could be voluntarily relaxed. Of course the attempt to relax sometimes met with failure. A close relationship between spasm or relaxation to habit is suggested, since after habitual relaxation had been cultivated, the spasm generally failed to appear.

2. When the subject ceased to relax, while the balloon remained in situ, the esophagus sometimes became tense again, but frequently it remained relaxed. The supine position seemed to favor relaxation, but usually did not of itself effect this result.

3. The lower smooth muscle portion of the esophagus behaved like the middle portion during relaxation.

4. If the tonus of the esophageal musculature may vary with that of the skeletal system, it would seem simplest to assume that esophageal relaxation proceeds through diminution of spinal impulses from skeletal muscles as these relax.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

I. Standard Basal Ration Bread and Experimental Methods

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From The University of Rochester School of Medicine and Dentistry

Received for publication February 13, 1925

Our earlier anemia experiments (9) convinced us that the amount of hemoglobin regeneration in the body could be modified at will by diet control. These experiments were *qualitative* in nature but indicated that an extended series of experiments would give valuable information relating to normal hemoglobin construction and pigment metabolism in normal and diseased conditions. Because of our conviction of the importance of general hemoglobin metabolism we examined all the experimental factors with considerable care with the hope that some of our experimental variables could be reduced to constants.

We attempted to insure a *constant and maximal stimulus* to the production of hemoglobin and red cells by a uniform anemia level maintained a constant from week to week. It is obvious that a severe anemia produced by two or three large hemorrhages will cause a maximal stimulus to hemoglobin and red blood cell production but if the hemoglobin level is permitted to rise toward normal it is very probable that this stimulus decreases in intensity. Our earlier experiments in this field have been concerned with simple anemia in dogs produced by two or three large hemorrhages at the beginning of the anemia period. The curve of regeneration of hemoglobin and red cells was then followed back to normal and the influence of various diet factors recorded.

These recent experiments on dogs tabulated below deal with a *constantly maintained severe secondary anemia.* An attempt is made to reduce the hemoglobin level to about 40 or 50 per cent and to maintain this anemia by frequent bleedings of calculated amounts. The bleeding samples are measured for total hemoglobin and we are able to state from week to week how much hemoglobin the body can produce over and above the maintenance factor which presumably is reasonably constant under these

[1] This work has been aided by a National Live Stock and Meat Board Fellowship of the National Research Council. We are indebted to the Rochester Packing Company for a liberal supply of certain meat products. We acknowledge with pleasure the friendly advice and assistance of Dr. E. B. Forbes and Dr. C. Robert Moulton.

conditions. It may be assumed that with this degree of anemia the stimulus to red cell and hemoglobin formation is a sustained maximum and this should test the value of any diet factors for hemoglobin manufacture in the body.

If we start with a 50 per cent hemoglobin anemia and after many months of continuous anemia finish the experiment with a 50 per cent hemoglobin level it seems safe to conclude that the various amounts of hemoglobin removed by bleeding week by week represent the maximal productive capacity of the body during the various diet periods. This *hemoglobin removed can be measured* with accuracy and the procedure is not open to objections which may be raised against any calculation of the *circulating hemoglobin.*

Objections can be raised to almost any *type of animal* used for such anemia experiments. Rabbits and guinea pigs are least suitable for this work as many of the food substances most potent in hemoglobin production (meat products) are foreign to their diet and unsuitable for utilization by their digestive tract.

Rats are in many respects excellent as their growth and metabolism factors are so well understood. Purified foods, amino acids and expensive synthetic chemical diet factors can be tested without unreasonable expense. But they have such small amounts of circulating blood and inaccessible veins that the measure of red cell and hemoglobin values is of necessity inaccurate and the measure of plasma volume an impossibility. These difficulties are recognized in part by Jencks (5).

To insure accuracy of determinations in an *anemic animal* one must obtain a small sample of blood by a needle in a vein or in the heart. One who doubts this statement need only follow a series of normal rats under constant laboratory conditions making repeated red cell and hemoglobin determinations over considerable periods of time using the usual technique. There are obvious objections to frequent cardiac puncture.

The dog in our opinion offers the best experimental opportunity for this work. Blood samples can be obtained easily from the veins and the circulating volume is large enough so that it is not disturbed by frequent sampling. Plasma volume determinations can be done easily and with considerable accuracy. It is obvious from the tables below that one will fall into error if the plasma volume is not known. Under certain conditions a rise in the hemoglobin values may be due to a shrinkage in plasma volume or a fall in hemoglobin to a plasma increase. Plasma volumes in anemic dogs (and presumably other anemic animals) are subject to much greater fluctuations than are the plasma volumes of normal dogs.

Dogs are omnivorous and on suitable diets can be kept in health over many months in spite of extreme experimental anemia. Our animals were born and raised in our kennels and will eat almost any sort of food mixture with relish and alacrity.

METHOD. Dogs were used in all these experiments. Practically without exception our dog colony has been healthy during all experimental periods and unless special note is made to the contrary normal conditions obtained throughout the experiments. These experimental animals were born and raised in the kennel and therefore accustomed to that place of residence, and to the simple procedures used for the removal of blood by venous puncture. The cages are large and comfortable and permit of much exercise. Not more than 12 dogs are kept in any room and every animal is kept at all times in its own cage. Cages are cleaned daily and the dog is transferred to an empty cage and care is taken that there is no access to any food elsewhere in the room during the cleaning period. Water is available in the cage at all times. The dog rooms are well ventilated by exhaust fans and a uniform temperature of 65 to 70°F. maintained by thermostatic control. We consider these conditions important for the successful conduct of a long anemia experiment and every care should be taken to maintain uniform temperature conditions day and night. These anemia animals are carefully isolated to prevent infection with distemper.

The dogs are fed once daily in the afternoon and the various food ingredients are so thoroughly mixed that the animals cannot pick out any single food ingredient which may be more to their taste. When bread is used in the mixture about equal parts of water are added to give a hash of suitable texture for thorough mixing. The various blood samples, blood volume determinations, etc., are done in the morning and the dogs are so familiar with this procedure that they are not at all disturbed by venous puncture and blood aspiration.

Our dogs after normal or resting periods have a hemoglobin level of 120 to 150 per cent. The desired *anemia level* is induced by three, four or more bleedings spaced at one or two-day intervals. Care must be used else too rapid bleeding and blood concentration may cause fatal shock. This anemia level of 40 to 50 per cent hemoglobin is then maintained as near a constant as possible by hemorrhages graded as to frequency and amounts by the readings of small samples taken at intervals each week for hemoglobin and hematocrit readings. Fluctuations of plasma volume must be taken into consideration in estimating the required bleeding in various experiments. It is obvious from the tables that fasting, diarrhea or intoxication may cause shrinkage of plasma volume in anemia dogs.

Hemoglobin pigment in grams is determined in every aspirated bleeding sample. Following every hemorrhage after an interval of 48 hours there are determined the hemoglobin and red cell hematocrit. Blood plasma volume and red cell counts are done each week independent of these estimations of hemoglobin and red cell hematocrits. No blood volume determinations are made less than 48 hours following any hemorrhage. It

should be noted here that the small samples of blood taken one or two times each week to determine the hemoglobin level and red cell hematocrit are not measured quantitatively for hemoglobin and therefore are not included in the tables. This hemoglobin amounts to 1 or 2 grams per week and is a constant. With very rapid blood regeneration it may be necessary to remove three or four of such samples but the amount of hemoglobin removed is insignificant in relation to total hemoglobin regeneration under such conditions,—for example, 30 to 50 grams of hemoglobin.

Blood plasma volume is measured by a method described in detail recently (4). This method utilizes the dilution of an inert red dye (brilliant vital red) by the circulating plasma volume.

Hemoglobin is measured in the form of acid hematin colorimetrically against a known standard as described recently by one of us (7). This standard at 100 per cent contains 13.8 grams of hemoglobin.

Red cell hematocrit values are obtained from a sample of blood taken into a 1.6 per cent solution of sodium oxalate (isotonic to dog's blood) in graduated, calibrated 15 cc. centrifuge tubes. These tubes are centrifugalized at 2200 revolutions per minute for 30 minutes which experience has shown gives uniform and maximal packing of cells.

Red cell and white blood cell counts are made by familiar technique from samples of blood taken from the jugular vein. White cell counts are not included in our tables but are recorded in footnotes when marked deviation from normal is noted.

Color index is obtained in the usual manner from the red cell count and hemoglobin figures.

Hemoglobin index in these papers means the figure obtained by dividing the hemoglobin per cent by the red cell hematocrit. This figure may give information of value as to the saturation of the red cell stroma with hemoglobin.

The hemoglobin index in the tables is determined from the last sample of blood taken at the end of each week. A blood volume and red cell count may or may not be done on this same day. In the tables the hemoglobin, red cell hematocrit and hemoglobin index always agree as they are determined from the same sample of blood. The red cell count and *color index* may have been done at the same time or two or three days before the hemoglobin index sample was taken and there may have been a bleeding hemorrhage between these determinations. For this reason the color index may appear to be incorrectly calculated from the hemoglobin figures in the tables.

Bread ingredients. The flour employed is ordinary bread flour, Veteran brand. The bran is that of the wheat kernel and is slightly coated with molasses. The salt mixture is that of McCollum and Simmonds (4) minus the ferric citrate. The cod liver oil is a commercial pharmaceutical pro-

duct not highly refined. Both the tomato and salmon represent medium grade canned products, the salmon being designated as "Alaska pink."

The *bread mixture* is prepared in the following manner: The bread flour, potato starch, salt mixture, cod liver oil, finely divided salmon crumbs, water and dissolved yeast are thoroughly mixed and permitted to stand over night at 70°F. The following morning the remaining ingredients are added, the entire mixture thoroughly beaten, kneaded, formed into loaves of even size and placed in pans lightly coated with cod liver oil. About 2 hours suffice to raise the bread the desired amount. The loaves are baked for a period of 45 minutes. When cool the bread is broken up into small fragments, dried in large shallow trays over radiators and is then ready for incorporation into various diets.

TABLE A

Bread (C) = Standard bread

INGREDIENTS		PROTEIN	FAT	CARBOHYDRATES
	grams	*grams*	*grams*	*grams*
Wheat flour	12,000	1,240	125	8,480
Potato starch	6,000			5,400
Bran	2,000	300	86	1,080
Sugar	4,000			4,000
Cod liver oil	2,000		2,000	
Canned tomatoes	2,000	24	4	80
Yeast, compressed	454	55	2	96
* Salt mixture	120			
Water	7,500			
Total	36,074	1,619	2,217	19,136

Protein, 7.0 per cent.
Fat, 9.7 per cent.
Carbohydrate, 83.0 per cent.
Caloric value 4.12 per gram as fed.
* McCollum and Simmonds' salt mixture with ferric citrate omitted.

Experimental observations. Under the heading of method we describe various measures taken to ensure uniformity in the general experimental environment of the anemia animals; uniformity in general régime, room temperature, light and ventilation and exercise facilities. Excellent bodily condition and appetite are a necessity for the continuance of these long experiments.

The general experimental procedure is well illustrated by tables 1 and 2 extending over 32 and 26 weeks respectively. As a general rule 2-week bread periods alternate with 2-week periods of other food factors which may wholly replace the standard bread or supplement a partial bread ration as indicated under the diet periods. Careful study of the various

diet periods shows that these two dogs regenerated very little if any hemoglobin during bread periods, over and above the maintenance factor.

We do not wish to draw attention to the various diet factors which act favorably upon hemoglobin regeneration. Subsequent papers will supplement these data but the two tables given show a characteristic sequence of events and illustrate the relatively inert character of this basal ration bread as regards production of hemoglobin and red cells in the body.

In table 1 may be noted a number of interesting points some of which will be covered in detail in subsequent papers. Fasting periods of two weeks show an increase in red cells and hemoglobin over and above this main-

TABLE B

Bread (S) = Salmon Bread

INGREDIENTS		PROTEIN	FAT	CARBOHYDRATE
	grams	*grams*	*grams*	*grams*
Wheat flour	12,000	1,240	125	8,480
Potato starch	6,000			5,400
Bran	2,000	300	86	1,080
Sugar	3,000			3,000
Cod liver oil	1,000		1,000	
Canned tomatoes	2,000	24	4	80
Canned salmon	2,500	545	302	
Yeast, compressed	455	55	2	96
*Salt mixture	150			
Water	7,500			
Total		2,164	1,419	18,136

Protein, 10.0 per cent.
Fat, 6.5 per cent.
Carbohydrate, 83.4 per cent.
Caloric value 4.8 per gram as fed.
* McCollum and Simmonds' salt mixture with ferric citrate omitted.

tenance factor which shows that the body can use its own protein radicles to reconstruct the needed hemoglobin. Bread periods show very little if any hemoglobin production and the best control periods are at the first part of the table. This dog was on the experimental régime for two weeks on bread and two weeks on fasting, immediately preceding the periods shown in this table 1. This fore period eliminated any "carry over" hemoglobin factors which are stored in the body during favorable diet periods and used up during the first 2 or 3 weeks of the anemia period. It is obvious that beef muscle, brains, butter, gelatin and eggs are moderately favorable for hemoglobin production. Ox bile tablets are inert under these conditions.

TABLE 1

Dog 20-1 bull, female, adult. Experiment begins December 11, 1923

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 500	83	14.2	1,110	3.2	0.63	2.05	19.3	40	0
Bread (C) 500	95	14.1	1,064	3.8	0.54	2.00	20.4	41	0
Fasting	0	12.2	972	5.6	0.47	2.20	24.0	53	0
Fasting	0	11.0	846	3.6	0.54	1.69	23.1	39	19.4
Bread (C)	69	11.3	1,022	4.0	0.54	1.97	22.0	43	0
Bread (C)	73	11.5	995	4.3	0.48	1.83	22.5	41	0
Brains 300, br. (C) 300	100	11.4	1,008	3.7	0.62	1.86	24.5	46	0
Brains 300, br. (C) 300	100	12.2	956	4.1	0.71	1.96	25.8	51	18.6
Brains 200, br. (C) 400	100	12.5	976	3.7	0.68	2.04	24.5	50	0
Brains 300, br. (C) 400	100	12.9	955	4.7	0.54	1.86	21.1	39	29.1
Bread (C) 500	88	12.6	1,008	5.2	0.43	2.03	22.2	45	0
Bread (C) 500	88	11.7	914	5.4	0.49	2.14	24.7	53	0
Beef 200, Br. (C) 400	100	14.1	1,084	4.4	0.48	2.06	20.7	43	16.8
Beef 200, Br. (C) 400	100	15.2	1,082	5.3	0.50	1.96	20.7	41	13.2
Bread (C) 400	100	15.0	1,138	5.0	0.41	1.85	21.9	41	0
Bread (C) 400	100	14.5	935	4.5	0.51	2.15	21.4	46	0
Oxgall 6 T. br. (C) 400*	100	13.4	646	5.3	0.55	2.26	26.0	59	0
Oxgall 4 T. br. (C) 400*	47	13.0	850	5.0	0.48	2.50	19.3	48	0
Tripe 300, br. (C) 300	100	14.3	1,055	4.9	0.54	2.36	22.5	53	0
Tripe 300, br. (C) 300	100	15.7	1,208	4.1	0.55	2.21	20.4	45	12.5
Bread (C) 450*	100	14.9	774	5.3	0.50	2.38	18.4	44	16.0
Bread (C) 450*	100	15.1	872	3.7	0.53	2.27	17.2	39	0
Butter 50, br. (C) 400	100	15.1	860	5.4	0.50	2.38	16.3	39	15.2
Butter 50, br. (C) 400	100	15.3	838	4.7	0.64	2.07	19.4	40	14.6
Bread (C) 450	100	14.8	858	4.6	0.51	2.31	20.3	47	0
Gelatine 50, br. (C) 400	100	15.0	845	5.0	0.55	2.44	22.4	55	12.9
Gelatine 50, br. (C) 400	100	15.3	850	3.7	0.59	2.31	18.8	43	10.7
Bread (C) 450	99	15.5	795	4.5	0.48	2.27	19.1	43	0
Bread (C) 450	93	15.5	795	5.7	0.46	2.50	21.2	53	14.2
Eggs 150, br. (C) 400	94	15.4	818	4.9	0.54	2.50	19.2	48	10.9
Eggs 150, br. (C) 400	99	15.8	866	4.0	0.52	2.38	17.5	42	0
Bread (C) 450	100	15.8	972	5.0	0.41	2.00	20.4	41	0

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Diarrhea present.

TABLE 2

Dog 19-104 bull mongrel, male, adult. Experiment begins October 17, 1923

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
Food, grams per day:	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Bread (C) 350	28	10.4	576	3.8	0.75	2.35	24.2	57	**0**
Bread (C) 350	56	10.4	730	4.0	0.70	2.42	23.2	56	**0**
Liver (beef) 400	100	10.8	672	4.9	0.87	2.24	38.2	86	**10.9**
Liver (beef) 400	100	10.7	618	5.5	0.60	2.22	29.5	66	**69.3**
Bread (C) 350	56	10.4	640	4.1	0.66	2.22	24.3	54	**27.9**
Bread (C) 350	69	10.1	632	3.6	0.67	2.08	23.2	48	**0**
RBC 10, bread (C) 350*	95	10.9	689	4.4	0.70	2.31	26.5	61	**0**
RBC 10, bread (C) 350*	93	11.1	753	4.0	0.54	1.88	22.9	43	**13.9**
RBC 10, bread (C) 350*	100	11.2	772	4.6	0.51	1.93	24.3	47	**0**
RBC 10, bread (C) 350*	100	11.7	706	5.9	0.43	1.85	23.1	43	**12.9**
Hb. perit. 2, br. (C) 350†	98	11.8	694	5.5	0.46	1.97	25.8	51	**0**
Hb. perit. 5, br. (C) 350†	68	11.7	752	5.1	0.53	1.96	22.2	44	**29.0**
Bread (C) 350	66	11.3	718	5.5	0.52	1.97	21.4	42	**15.1**
Bread (C) 350	61	11.1	650	5.2	0.45	1.88	24.7	46	**0**
Bread (C) 350	60	10.6	662	4.5	0.53	2.14	22.3	48	**0**
Bread (C) 350	45	10.2	616	5.1	0.54	1.98	21.2	42	**13.2**
Salmon 200, br. (C) 300	94	11.4	744	4.1	0.51	2.05	20.2	42	**0**
Salmon 200, br. (C) 300	85	11.4	732	4.7	0.40	1.86	20.4	38	**0**
Salmon 200, br. (C) 300	38	11.8	814	4.1	0.44	1.80	20.3	36	**0**
Heart (beef) 300, br. (C) 200	88	12.3	723	5.2	0.47	2.05	23.7	49	**0**
Heart (beef) 300, br. (C) 200	98	12.5	776	6.4	0.54	2.40	20.5	49	**17.9**
Bread (C) 350	22	10.9	638	5.1	0.52	2.22	23.9	53	**0**
Egg alb. 150, br. (C) 250	29	10.0	625	3.1	0.48	1.90	15.6	30	**11.5**
Liver 100, br. (C) 250	100	11.1	606	4.0	0.65	2.50	20.7	52	**0**
Liver 100, br. (C) 250	100	11.2	646	5.5	0.62	2.42	22.1	53	**28.5**
Bread (C) 350	81	10.9	606	5.0	0.62	2.20	16.9	37	**15.2**
Bread (C) 350	14	9.7	544	3.5	0.52	2.00	18.1	36	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* RBC 10 = 10 grams daily of whole red cells (beef) washed, dried and powdered.

† Hb. perit. = hemoglobin (dog) given intraperitoneally aseptically about 2 and 5 grams each day to a measured total of 12 and 34 grams respectively each week.

We observe further in table 1 that there are significant fluctuations in blood *plasma volume*. Intoxication, diarrhea and fasting induce a distinct shrinkage of plasma volume. The feeding of fresh cooked tripe is associated with a considerable increase of plasma volume. The color index shows a fall from normal figures of 0.90 at the start to 0.50 and 0.40 during some later periods. The hemoglobin index (hemoglobin per cent divided by red cell hematocrit per cent) shows interesting fluctuations discussed below.

Table 2 is much like table 1 and the control bread diet periods show very little hemoglobin and red cell regeneration. Most of the hemoglobin removed during bread periods is due to a "carry over" of parent hemoglobin

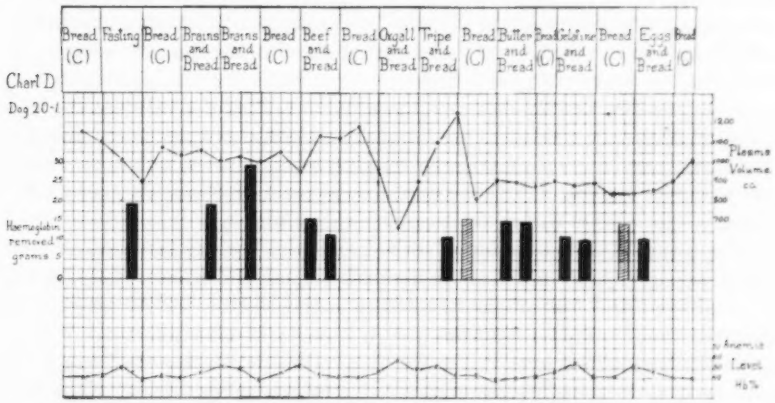

Chart D (Dog 20-1) illustrates in graphic form a series of reactions due to various diet periods. The upper curve shows the fluctuation of blood plasma volume from week to week. The lowest curve shows the anemia level in terms of hemoglobin percent which we attempt to maintain as near a constant as possible. The solid black columns represent grams of hemoglobin removed each week during specified diet periods. The cross hatched columns represent grams of hemoglobin removed in the weeks subsequent to the responsible diet—that is the "carry over" factor resulting from a favorable diet and disclosed in the week or weeks of unfavorable diet.

factors referable to the preceding favorable diet period, for example, the first liver feeding period shows a production of 80 grams of hemoglobin during the two weeks and in the following week there is a production of 28 grams which obviously is not due to the bread but to the preceding liver feeding. The contrast between liver and fish feeding is very striking. On several occasions this dog ate the bread mixture very poorly, consuming only $\frac{1}{4}$ of the food for each day. This necessitated changing to diets which were more to this dog's taste. During limited food intake we note a shrinkage of plasma volume as in table 1.

DISCUSSION. The *basal ration*[2] is of considerable importance for anemia experiments of this type. This food must be adequate to maintain the anemic dog in health for many months. It must be palatable and yet permit of minimal hemoglobin and red cell regeneration over and above the maintenance factor. We have used a considerable variety of food mixtures in our various anemia experiments, some published and many unpublished. Bakers' bread, cooked rice and potato, milk and cereals have been used in various combinations and proportions. These food substances are all unfavorable food factors for hemoglobin production in anemia. We experimented with a variety of bread mixtures cooked in the laboratory and finally came to the two types of bread described and tabulated above as best suited for our work. This *basal ration* bread is adequate to maintain dogs in health over long periods of time (months), and is used in the control periods alternating with or combined with other food factors or drugs to be tested.

This bread is usually consumed readily but some dogs do not eat the amount necessary to maintain a constant weight. Such animals may eat the bread completely if a few grams of salmon or beef muscle or meat extract are added to the bread. Such additions are noted in the tables and do not modify appreciably the production of hemoglobin.

The term "*maintenance factor*" of red cells and hemoglobin is often used in these papers and indicates the hemoglobin and red cells used up by the general wear and tear of the circulation in the body. Unfortunately this *maintenance factor* is unknown but in this type of experiment with a constant hemoglobin level it is probably a relatively constant factor. How closely can we guess at the value of this maintenance factor? The best work on the normal life cycle of the red cell in human beings is that of Ashby (1), whose work indicates that red cells may endure for 30 days or longer the vicissitudes of circulatory existence. If we may draw a parallel with the dog we may assume for the sake of argument that the dog may use up about 3 per cent of his red cells each day or about 20 per cent per week. If we assume a dog weight of 10 kilos and the hemoglobin level of 50 per cent we may calculate this maintenance factor. A 10-kilo dog might average about 800 cc. total blood volume with this degree of anemia. Therefore his total hemoglobin would be $8 \times 13.8 \times 0.50 = 55.2$ grams hemoglobin. If his maintenance factor is 20 per cent per week we arrive at 11.5 grams hemoglobin as the average for a 10-kilo dog. Ashby's work indicates that the human cells may be considerably longer lived than has been indicated and of course the life cycle of dog's corpuscles may be longer or shorter than the human. For example, we know that dog's red cells are much more fragile than those of many other animals but this need not mean a shorter life.

[2] Some of the preliminary work with the standard bread ration was done at the Hooper Foundation for Medical Research, University of California Medical School.

We may recall again at this point that *fasting* dogs conserve from their body protein katabolism certain elements which they build into hemoglobin and red cells, not only enough to make up this *maintenance fraction* but several grams of hemoglobin per week over and above that factor—truly a remarkable example of careful internal body conservation of necessary elements from among the protein building stones set free during fasting katabolism. Yet, it may lead to misinterpretation to speak of the hemoglobin as being "parasitic" (3) upon the body tissues, for during fasting anemia periods there is no more destruction of body protein than during simple fasting periods without anemia. The body is very careful to conserve the parts of broken down protein which can be recast into hemoglobin but the body does not break down its protein for the purpose of furnishing hemoglobin no matter how intense may be the anemia stimulus.

We speak of a "carry over" from a favorable diet period into the control or bread periods. By this is meant that a dog on a favorable diet will store somewhere in its tissues or organs the ingredients for hemoglobin and red cell construction which later during unfavorable diet periods will be drawn upon to manufacture hemoglobin and red cells. Table 2 illustrates this point admirably and we see the liver diet period with its high hemoglobin production followed by an after control period during which a large amount of hemoglobin is formed. This *carry over* lasts rarely more than 2 weeks, and usually this reserve is largely exhausted during the control week following the favorable diet as illustrated in table 2.

In our earlier experiments (9) we came to recognize a *reserve factor* which all normal dogs possess, giving them power to regenerate large amounts of hemoglobin in the first week's period following a simple anemia due to two or three large hemorrhages. Note below in table 24, dog 21–67. The anemia experiments on rats reported by Jencks (5) indicate that these animals possess a similar reserve which does not seem to be recognized by Jencks. It would be most interesting to be able to study the curve of hemoglobin and red cell regeneration in these rats from day to day on the various diets but these figures are not given. Her tables give ample evidence of the difficulty of obtaining reasonable accuracy in blood counts and hemoglobin determinations in blood samples obtained from the rat by the usual technique. We now know that the dog on any *favorable diet* will store this reserve, which can be drawn upon later under anemic conditions with unfavorable diet intake. It might be argued that this reserve exists in the marrow in the form of finished red cells ready for an emergency but the evidence of our anemia experiments is against this explanation. Such a reserve could scarcely endure the conditions of a prolonged secondary anemia yet we see under severe anemia conditions this reserve storage on almost any favorable diet. Subsequent unfavorable diet periods invariably demonstrate its existence. We may assume for

the present that it is stored in the body, probably largely in the liver, perhaps in the kidneys, spleen, marrow and muscles and in the form of parent substances at present unknown but readily transformed into cells and hemoglobin under conditions of anemia stress. It is at least possible that such factors may be stored in the heart and skeletal muscles against the needs of these muscles for production and maintenance of muscle hemoglobin.

In another place (2) we have discussed the variations in plasma volume which follow hemorrhages, showing that there is an increase in plasma as the red cells are depleted by bleeding. The normal dog has a plasma volume of about 5 per cent of body weight and these anemic dogs have a plasma volume of about 7 per cent. We note in these anemia experiments that the plasma volume is much more labile than in a normal dog. Tables 1 and 2 show that fasting and diarrhea will cause a prompt shrinkage in plasma volume while a rapid increase of weight on favorable diet will cause an equally prompt rise in plasma volume. It is much more necessary to know the plasma volume of an anemic dog than that of a normal animal to guard against erroneous conclusions, drawn from hemoglobin, red cell hematocrit and red cell count figures.

SUMMARY

A severe secondary anemia is produced in dogs and this anemia level is maintained as near a constant as possible by suitable bleedings. By this means it is believed that the *stimulus to hemoglobin production* will be a *sustained maximum* and the *maintenance replacement factor* for hemoglobin and red cells as nearly a constant as possible.

Two standard basal rations are described which furnish complete food requirements for the adult dog even under severe anemia conditions and yet permit of minimal hemoglobin and red cell regeneration over and above the unknown maintenance factor.

The efficiency of other diet factors and drugs can then be tested by alternating with control bread periods.

The advantages and disadvantages of different animals for anemia experiments are discussed. The various methods, laboratory procedures, care of animals, etc., are described in detail.

There are certain *important differences* in diet and hemoglobin reactions in these long continued anemia experiments, contrasting with our short anemia experiments formerly published (9). These differences are important and will be brought out in the later papers of this series.

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BLOOD REGENERATION IN SEVERE ANEMIA

II. Favorable Influence of Liver, Heart and Skeletal Muscle in Diet

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Received for publication February 13, 1925

We have combined these three food factors in this paper because in our earlier experiments with simple anemia of short duration these food factors were most potent in effecting a rapid production of hemoglobin and red cells with prompt return to a normal blood picture.

Liver feeding in these severe anemias remains the most potent factor for the sustained production of hemoglobin and red cells as indicated in various tables. This favorable and remarkable reaction is invariable in our dog experiments no matter how long continued the anemia level, no matter how unfavorable the preceding diet periods may be and regardless of the substances given with the liver feeding.

Striated muscle feeding—heart or skeletal—does not give the same uniformly favorable reaction as liver feeding in severe anemia. There seems to be a distinct difference from the short anemia experiments (2) in which muscle feeding gave more favorable reactions. Muscle feeding in severe anemia dogs gives a favorable reaction in many cases and only a moderate reaction in others. Again the heart muscle seems to be somewhat more potent in hemoglobin and red cell production as compared with skeletal muscle.

Our earlier experiments with short anemia periods and favorable meat reaction were usually associated with bread and milk feeding preceding the meat periods. It is possible that these different diets preceding the meat feeding may explain some of these differences in experimental reaction. The severity of the anemia may also be a factor as is the case in the experiments with iron presented in the next paper. We have noted (2) that in experiments with long periods of unfavorable diet and consequent slow hemoglobin regeneration the subsequent meat diet periods gave much less hemoglobin and red cell regeneration than did liver feeding periods. It was suspected that these unfavorable diets had depleted some reserve in the body which would normally contribute to subsequent hemoglobin regeneration.

One may suspect that the *muscle hemoglobin* may be concerned in the favorable reaction noted with meat feeding. This point will be taken up again in a subsequent paper dealing with hemoglobin feeding and injection. It may be pointed out here that the usual diet sample of 300 grams cooked skeletal muscle contains when fresh about 3.0 grams of muscle hemoglobin as determined by our method. This amount of hemoglobin alone is sufficient to cause some hemoglobin and red cell production when added to a bread ration, but there probably are other more important factors—for example, beef heart may be more potent in hemoglobin and red cell regeneration (table 21) yet it contains less muscle hemoglobin per 100 grams than does the usual skeletal muscle used in feeding.

EXPERIMENTAL OBSERVATIONS. The general methods, care of animals and other experimental details are given in the first paper of this series We give two tables similar to those of paper I which show the sequence of the diet periods from week to week during several months. Another type (tables 23, 24 and 25) groups the various experiments showing the influence of a single diet factor in several animals with necessary control fore and after periods.

Table 21 shows several interesting points. There is a notable difference in hemoglobin and red cell production during various diet periods of meat products, fish and fresh green vegetables. Meat products (beef liver, kidney and spleen) have a very favorable reaction while canned salmon and dried codfish are almost inert. Cooked green vegetables are only moderately favorable food factors for hemoglobin and red cell production in severe anemia. We are concerned in this table 21 with the details only of liver and beef heart feeding. This shows a truly remarkable reaction to the beef heart diet—the maximum reaction of which we have a record and the dog regenerated over 90 grams of hemoglobin as a result of two weeks' heavy feeding of cooked beef heart.

The general reaction of this dog to all food factors is maximal and she even forms a definite amount of hemoglobin each week on the basal bread ration. The average dog may form 1 or 2 grams of hemoglobin each week above his maintenance factor on bread feeding but this dog averages about 4 or 5 grams per week on the bread ration. This is a personal peculiarity not associated so far as we know with breed, sex or age. These actively regenerating dogs react almost always in maximal degree to all diet factors—their threshold reaction is higher than normal and is a sustained reaction which must be understood properly to interpret the experimental data.

We note that during diet periods of beef heart, liver or spleen there is an increase in the hemoglobin index. This is not a constant reaction in other dogs and more data may determine whether this reaction is frequent or exceptional. One may suspect that this reaction, indicating an increase

TABLE 21

Dog 18-114 bull, female, adult. Experiment begins February 11, 1924

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Salmon (canned) 300, br. (C) 300	99	12.6	797	4.9	0.59	2.05	20.8	43	**14.5**
Salmon (canned) 300, br. (C) 300	89	13.1	885	3.8	0.62	2.08	22.6	47	**0**
Salmon (canned) 300, br. (C) 300	91	12.9	716	4.9	0.49	1.96	24.5	48	**0**
Marrow and spl. br. (C) 450*	34	12.7	658	6.2	0.49	2.13	21.1	45	**13.7**
Marrow and spl. br. (C) 450	47	12.1	708	4.7	0.56	2.38	22.1	53	**0**
Heart (beef) 400, br. (C) 300	93	14.3	752	5.3	0.64	2.34	28.9	68	**0**
Heart (beef) 400, br. (C) 300	94	14.8	762	5.2	0.69	2.50	23.0	58	**63.3**
Bread (C) 450	26	13.4	676	3.9	0.74	2.10	16.9	35	**32.3**
Egg yolk 85, br. (C) 350	64	13.4	774	3.5	0.77	2.42	18.5	45	**10.0**
Egg yolk 85, br. (C) 350	73	13.0	712	4.5	0.47	2.05	20.6	42	**0**
Pancreas (calf) 200, br. (C) 300	98	13.7	642	4.4	0.52	2.19	18.8	41	**9.9**
Pancreas (calf) 200, br. (C) 300	100	14.1	724	4.9	0.55	2.31	20.5	47	**12.1**
Bread (C) 400	38	13.1	706	4.9	0.53	2.38	18.1	43	**12.7**
Dried codfish 100, br. (C) 300	65	12.8	654	3.3	0.51	1.99	17.0	34	**8.4**
Spleen (calf) 200, br. (C) 300	100	12.6	844	4.2	0.59	2.40	18.0	43	**10.7**
Spleen (calf) 200, br. (C) 300	100	13.2	692	5.8	0.48	2.59	21.3	55	**13.3**
Bread (C) 400	82	12.9	627	5.2	0.63	2.46	19.7	48	**22.4**
Bread (C) 400 salmon 50	79	12.9	656	4.4	0.58	2.61	16.9	44	**13.5**
Spinach 200, br. (C) 300	71	12.6	813	4.7	0.48	2.07	20.2	42	**8.9**
Spinach 200, br. (C) 300	60	12.1	742	5.2	0.40	2.14	19.6	42	**0**
Lettuce 150, br. (C) 350†	80	12.1	734	6.3	0.41	2.24	18.9	42	**14.1**
Lettuce 150, br. (C) 350	77	12.0	725	5.4	0.44	2.24	19.7	44	**11.3**
Bread (C) 400, salmon 25	58	11.5	822	4.7	0.42	1.97	20.0	39	**0**
Liver (beef) 450	100	12.2	740	5.2	0.61	2.31	27.5	63	**21.3**
Liver (beef) 450	100	12.5	673	3.4	0.71	2.22	21.8	48	**38.1**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Marrow and spl. = dried, powdered marrow and spleen extract, 2 capsules per day, furnished by Dr. C. D. Leake, 50 grams beef heart added to diet.

† Thirty-five grams salmon added to diet.

of hemoglobin in the red cell matrix measured in *cubic centimeters* of packed red cells, suggests a greater speed of hemoglobin regeneration over stroma regeneration during these diet periods.

Table 22 contains many data of interest. This dog did not eat the bread mixture as well as usual, consequently a considerable number of meat products are used which perhaps explains the sustained high values of the hemoglobin index. During complete or partial fasting periods we observe considerable shrinkage of the plasma volume and by contrast a great increase in plasma volume during a diet period of tripe. This great plasma volume increase from 467 cc. to 796 cc. causes a corresponding decrease in the hemoglobin values without any bleeding—an excellent illustration of the need for plasma volume determinations in these anemia experiments.

The two liver diet periods (table 22) show a notable difference in hemoglobin regeneration. The first period with 350 to 400 grams of liver daily for two weeks shows a total hemoglobin regeneration of about 85 grams including the "carry-over" in the after period. The second period with 300 grams of liver and 300 grams of bread daily for 2 weeks shows less hemoglobin or about 45 grams including the "carry over." During this second period there was a notable gain in weight of 3 kilos, following a fasting period. We have other data (table 24) which indicate that during periods of rapid increase in body weight the expected hemoglobin regeneration may not be recorded. This may indicate that such gains in weight make a demand upon diet factors which otherwise would go into the formation of hemoglobin and red cells. The first two weeks period (table 22) shows an expected carry over due to the preceding kennel diet. This reserve factor stored during favorable diet periods often amounts to more than this total of hemoglobin and at times requires three weeks before the reserve is completely exhausted. We note a favorable reaction under these conditions to administration of iron in the form of Blaud's pills and this reaction will be discussed in detail in the next paper. A diet of beef kidney gives a favorable hemoglobin regeneration but the figures are slightly misleading. We note that 25 grams of hemoglobin were removed during the first week of kidney feeding but some considerable part of this is really due to a "carry over" from the after period of iron feeding—note the hemoglobin level of 76 per cent before the kidney feeding.

Table 23 brings together some characteristic reactions due to beef liver feeding. Varying amounts of liver consumption bring about as a rule corresponding increases in hemoglobin and red cell regeneration. The maximum regeneration of hemoglobin is about 100 grams due to a two weeks diet period of liver feeding (400 grams per day—dog 19–104). About the same amount of liver feeding in a larger dog gives only 70 grams of hemoglobin regeneration. A smaller amount of liver (250 grams liver

TABLE 22

Dog 20-104 bull, male adult. Experiment begins October 10, 1923

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 350	23	8.4	525	4.2	0.77	2.38	27.0	64	**0**
Bread (C) 350	52	8.0	482	2.9	1.15	2.38	28.1	67	**26.8**
Liver (beef) 350	100	8.3	527	3.8	0.97	2.31	32.0	74	**24.9**
Liver (beef) 400	100	8.7	532	5.6	0.87	2.22	44.0	98	**17.0**
Bread (C) 350	80	9.1	517	5.0	0.80	2.25	35.5	80	**41.5**
Bread (C) 350	81	8.9	548	2.9	0.82	2.27	20.9	47	**15.4**
Fasting		7.6	458	3.5	0.74	2.26	22.7	51	**0**
Fasting		6.8	461	4.4	0.59	1.87	27.5	52	**13.3**
Liver (beef) 300, br. (C) 300	89	9.0	628	4.4	0.66	2.17	26.7	58	**0**
Liver (beef) 300, br. (C) 300	100	9.9	704	5.2	0.67	2.03	25.5	52	**39.3**
Bread (C) 350	100	9.6	670	4.6	0.56	2.03	25.6	52	**0**
Bread (C) 350	96	9.9	639	5.6	0.59	2.14	21.1	45	**17.0**
Chloroform, br. (C) 350*	35	9.0	581	4.7	0.49	2.07	22.4	46	**0**
Chloroform, br. (C) 350	41	9.2	542	4.6	0.62	2.03	20.4	41	**9.6**
Blaud's pills 2 T. br. (C) 350	65	9.0	538	5.2	0.55	1.97	22.4	44	**11.7**
Blaud's pills 2 T. br. (C) 350	62	8.8	493	5.5	0.62	2.08	25.7	53	**17.0**
Bread (C) 350	45	8.4	480	4.8	0.69	2.22	23.0	51	**16.6**
Bread (C) 350	35	8.0	442	5.2	0.73	2.34	32.4	76	**0**
Kidneys (beef) 300, br. (C) 300	100	10.7	652	5.0	0.80	2.31	25.0	58	**25.5**
Kidneys (beef) 300, br. (C) 300	83	9.5	535	3.2	0.81	2.27	22.9	52	**28.5**
Bread (C) 300	48	9.6	562	3.4	0.76	2.19	23.6	52	**0**
Bread (C) 300	39	9.1	467	3.1	0.72	2.36	18.9	45	**11.7**
Tripe 200, br. (C) 300	87	10.1	558	3.3	0.84	2.56	21.7	56	**0**
Tripe 200, br. (C) 300	61	10.0	796	4.0	0.52	2.38	17.5	42	**12.2**
Bread (C) 350	19	9.3	454	3.5	0.75	2.45	15.5	38	**10.8**
Pancr. mixt. 200, br. (C) 250†	97	10.2	677	2.6	0.52	2.05	13.2	27	**0**
Pancr. mixt. 200, br. (C) 250	73	10.2	564	3.3	0.61	2.33	17.3	40	**0**
Egg yolk 85, br. (C) 300	48	9.7	436	5.1	0.59	2.34	16.7	39	**10.8**
Egg yolk 85, br. (C) 300	62	9.7	495	3.8	0.51	2.10	18.4	39	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Chloroform 1 cc. in olive oil and milk given by stomach tube once at beginning of each week.

† Pancr. mixt. = mixture of various amounts of calves' pancreas and thymus, approximately equal parts.

TABLE 23

Beef liver feeding

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Dog 19-104, bull mongrel, male, adult, from table 2									
Food, grams per day:									
Bread (C) 350	28	10.4	576	3.8	0.75	2.35	24.2	57	**0**
Bread (C) 350	56	10.4	730	4.0	0.70	2.42	23.2	56	**0**
Liver (beef) 400	100	10.8	672	4.9	0.87	2.24	38.2	86	**10.9**
Liver (beef) 400	100	10.7	618	5.5	0.60	2.22	29.5	66	**69.3**
Bread (C) 350	56	10.4	640	4.1	0.66	2.22	24.3	54	**27.9**
Bread (C) 350	69	10.1	632	3.6	0.67	2.08	23.2	48	**0**
Dog 21-64 bull mongrel, female, adult from table 32									
Food, grams per day:									
Bread (C) 450	46	14.1	818	3.4	0.59	2.36	17.0	40	**14.0**
Bread (C) 450	64	14.5	966	3.7	0.49	2.38	15.2	36	**0**
Liver (beef) 450	100	14.5	866	5.2	0.77	2.22	35.9	80	**0**
Liver (beef) 450	100	14.0	747	5.6	0.86	2.24	43.3	97	**47.3**
Bread (C) 450	67	14.5	906	3.8	0.78	2.34	25.2	59	**26.6**
Bread (C) 450	100	14.8	894	3.9	0.70	2.38	23.0	55	**0**
Dog 16-160 bull mongrel, male adult									
Food, grams per day:									
Bread (C) 400	100	11.4	702	3.5	0.75	2.38	21.9	52	**0**
Bread (C) 400	68	11.1	705	3.3	0.67	2.31	19.2	44	**13.4**
Dext. 25, cane sugar 75*		10.2	658	3.2	0.67	2.14	20.1	43	**0**
Dext. 25, cane sugar 75		9.6	674	3.2	0.72	2.19	21.1	46	**0**
Liver 250, bread (C) 250	100	11.0	718	4.1	0.70	2.31	24.6	57	**0**
Liver, 250, bread (C) 250	100	12.2	814	3.5	0.76	2.34	22.6	53	**23.9**
Dext. 25, cane sugar 75*		10.5	656	3.8	0.80	2.32	26.4	61	**12.5**
Dext. 25, cane sugar 75		9.6	620	3.6	0.66	1.83	26.0	48	**0**
Bread (C) 350	100	10.3	719	3.8	0.66	2.14	23.4	50	**0**
Bread (C) 350	74	9.9	720	4.4	0.56	2.06	23.8	49	**0**

TABLE 23—*Concluded*

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
Dog 19-104 bull mongrel, male, adult, from table 2									
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 350	22	10.9	638	5.1	0.52	2.22	23.9	53	**0**
Egg alb. 150, bread (C) 250	29	10.0	625	3.1	0.48	1.90	15.6	30	**11.5**
Liver 100, bread (C) 250	100	11.1	606	4.0	0.65	2.50	20.7	52	**0**
Liver 100, bread (C) 250	100	11.2	646	5.5	0.62	2.42	22.1	53	**28.5**
Bread (C) 350	81	10.9	606	5.0	0.62	2.20	16.9	37	**15.2**
Bread (C) 350	14	9.7	544	3.5	0.52	2.00	18.1	36	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Cane sugar 75 grams, dextrose 25 grams dissolved in 250 cc. water and given daily by stomach tube.

and 250 grams bread) effects only about 35 grams of hemoglobin regeneration but we note (dog 16–160) that this followed a fasting period. There was a considerable increase of weight during this liver-bread period and we have noted elsewhere that such gains of weight may diminish the expected amount of hemoglobin regeneration.

Table 24 shows some characteristic reactions to beef heart feeding. We have referred to the remarkable maximal regeneration of hemoglobin and red cells following beef heart feeding in table 21. These two experiments (table 24) are representative of the usual reaction and we observe a favorable result but nothing like as striking as the usual reaction to liver feeding. Dog 21-67 shows an initial "carry over" of about 75 grams of hemoglobin during the first three weeks subsequent to the normal period and kennel diet. This is an unusually large carry over reserve and may be explained in large part by a liberal kennel diet of dog biscuit and liver.

Table 25 indicates that beef muscle is somewhat less potent for hemoglobin and red cell regeneration than is beef heart. There are some individual differences illustrated in table 25. The general reaction to heavy meat feeding is excellent and the rapid gain in weight may call on the diet intake for substances which might otherwise go into hemoglobin and red cell formation. We note in the two experiments showing no apparent hemoglobin regeneration that there is a notable increase in plasma

volume with the body weight increase. The red cell hematocrit not showing a decrease we may safely conclude that the circulating hemoglobin and red cell volume have been definitely increased. This means an actual increase of hemoglobin within the body which is to be attributed to the

TABLE 24

Beef heart feeding

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
Dog 19-104 bull mongrel, male, adult from table 2									
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Salmon 200, br. (C) 300	94	11.4	744	4.1	0.51	2.05	20.2	42	0
Salmon 200, br. (C) 300	85	11.4	732	4.7	0.40	1.86	20.4	38	0
Salmon 200, br. (C) 300	38	11.8	814	4.1	0.44	1.80	20.3	36	0
Heart 300, br. (C) 200	88	12.3	723	5.2	0.47	2.05	23.7	49	0
Heart 300, br. (C) 200	98	12.5	776	6.4	0.54	2.40	20.5	49	17.9
Bread (C) 350	22	10.9	638	5.1	0.52	2.22	23.9	53	0
Egg alb. 150, br. (C) 250	29	10.0	625	3.1	0.48	1.90	15.6	30	11.5
Dog 21-67 bull mongrel, male, adult									
Food, grams per day:									
Bread (C) 400, heart 50	30	11.0	528	2.6	1.23	2.73	23.4	64	39.7
Bread (C) 400, heart 50	53	10.8	512	3.6	0.94	2.00	18.5	37	29.4
Bread (C) 400, heart 50	70	10.7	465	3.6	0.79	2.27	19.0	43	11.0
Heart 200, br. (C) 300	93	11.9	687	3.4	0.79	2.26	16.8	38	11.0
Heart 200, br. (C) 300	88	12.5	603	4.2	0.75	1.88	19.0	36	17.2
Codfish 100, br. (C) 300	91	11.6	585	3.1	0.68	2.19	19.3	42	0
Codfish 100, br. (C) 300	77	11.5	657	4.6	0.57	2.22	16.2	36	9.7
Codfish 100, br. (C) 300	72	11.5	667	3.5	0.53	2.44	15.2	37	0

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

meat feeding. However, we observe in the complete table of this experiment (table 31, paper III) that preceding periods of iron feeding gave a high hemoglobin and red cell production and a subsequent liver feeding the usual large increase in hemoglobin and red cell volume. Other meat experiments are given below (table 23, paper III).

TABLE 25

Beef muscle feeding

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Dog 20-1 bull, female, adult from table 1									
Food, grams per day:									
Bread (C) 500	88	12.6	1,008	5.2	0.43	2.03	22.2	45	**0**
Bread (C) 500	88	11.7	914	5.4	0.49	2.14	24.7	53	**0**
Beef 200, br. (C) 400	100	14.1	1,084	4.4	0.48	2.06	20.7	43	**16.8**
Beef 200, br. (C) 400	100	15.2	1,082	5.3	0.50	1.96	20.7	41	**13.2**
Bread (C) 400	100	15.0	1,138	5.0	0.41	1.85	21.9	41	**0**
Bread (C) 400	100	14.5	935	4.5	0.51	2.15	21.4	46	**0**
Dog 20-71 bull, male adult from table 31									
Food, grams per day:									
Bread (C) 500	56	11.4	732	5.3	0.47	2.11	23.7	50	**0**
Bread (C) 500	55	11.1	683	4.6	0.47	1.96	21.9	43	**13.5**
Beef 300, br. (C) 300	100	13.6	892	4.5	0.57	2.19	23.4	51	**0**
Beef 300, br. (C) 300	100	15.0	984	4.1	0.56	2.15	21.3	46	**0**
Beef 300, br. (C) 300	100	16.0	1,030	4.6	0.55	2.31	22.1	51	**0**
Bread (C) 500	100	15.5	966	5.9	0.44	2.14	16.0	34	**8.8**
Bread (C) 500	63	15.1	839	4.2	0.50	1.94	21.6	42	**0**
Dog 24-5 bull, male young adult									
Food, grams per day:									
Bread (C) 400	86	10.1	792	4.5	0.44	1.57	25.4	40	**0**
Bread (C) 400	77	10.1	748	5.2	0.47	1.91	25.6	49	**0**
RBC 5, bread (C) 400	88	10.4	832	4.7	0.48	1.95	23.2	45	**8.4**
RBC 5, bread (C) 400	88	10.5	760	5.9	0.46	1.88	23.4	44	**12.0**
Bread (C) 400	62	10.2	716	6.4	0.41	1.74	18.4	32	**10.3**
Bread (C) 400	60	10.0	674	4.6	0.45	1.92	21.7	42	**0**
Beef 400, br. (C) 300	100	12.5	909	4.7	0.47	1.94	22.7	44	**0**
Beef 400, br. (C) 300	100	13.8	982	5.4	0.41	1.84	24.2	44	**0**
Bread (C) 400	100	13.4	936	4.9	0.48	1.96	24.1	47	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

DISCUSSION. How may we explain this remarkable productive reaction of hemoglobin and red cells due to liver feeding? In our opinion this is evidence that the *body stores in the liver* parent substances which are used in the construction of hemoglobin and red cells. This confirms our belief gained from work with bile fistula dogs (1) that the liver is concerned fundamentally with pigment metabolism not only of bile pigments and their parent substances but with the production and storage of some parent substances used in the manufacture of finished hemoglobin and red cells.

The "carry over" on favorable diets is well illustrated in table 23. The fore period of two weeks' bread shows little if any hemoglobin production. The two weeks' liver diet causes more or less abundant production of hemoglobin largely dependent upon the amount of liver fed daily. The after period of two weeks' bread feeding shows considerable production of hemoglobin—a "carry over" from the favorable liver diet period. This storage of hemoglobin parent substances during favorable periods is an invariable reaction which can readily be demonstrated during subsequent unfavorable diet periods when this reserve storage is drawn upon. The reserve is usually largely depleted during the first week of anemia and unfavorable diet but at times, especially following long periods of favorable diet without anemia, we may encounter a reserve storage requiring two or three weeks for exhaustion. Errors of interpretation easily arise unless this "carry over" reserve is clearly appreciated.

It may be argued that some of this "carry over" may be in the form of *immature* red cells which are being rapidly formed in the marrow. However, it is likely that more of the stroma is in such storage than is the hemoglobin, as it is well known that young red cells and parent forms have less hemoglobin than have the mature red cells. Moreover we can see in certain tables some evidence that the liver feeding is a little more favorable for hemoglobin than for red cell formation.

One need not be surprised to find that parent pigment substances are stored in striated muscle. This storage might be concerned with the emergency needs of these muscles to maintain their content of muscle hemoglobin.

SUMMARY

Beef liver feeding in severe anemia is associated with a maximal regeneration of hemoglobin and red cells. Abundant liver feeding for two weeks may cause the production of 90 to 100 grams of hemoglobin over and above the maintenance factor (table 23). In our opinion this indicates the storage within the normal liver of *parent pigment substances* and suggests that the liver is concerned with *pigment construction.*

Beef heart feeding is distinctly less favorable to hemoglobin and red cell regeneration, when compared with liver feeding.

Beef muscle (lean beef) is still less favorable for hemoglobin and red cell production than is beef heart. The reaction to striated muscle (heart and skeletal) feeding is subject to wide variations.

Beef muscle feeding appears to be more favorable to hemoglobin regeneration in our earlier *short anemia experiments* than in this severe anemia series.

During favorable diet periods even with attendant severe anemia the body stores away parent pigment substances. This storage is easily demonstrated by subsequent periods of unfavorable diet, whereupon this "carry over" surplus appears as excess hemoglobin and red cells. This storage probably involves both liver and bone marrow and perhaps spleen and muscles.

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BLOOD REGENERATION IN SEVERE ANEMIA

III. Iron Reaction Favorable—Arsenic and Germanium Dioxide Almost Inert

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Received for publication February 13, 1925

The history of anemia treatment with drugs is indeed a tale to make the judicious grieve. A multitude of drugs have been proposed, enthusiastically supported, then questioned and finally abandoned. Certain drugs appear to have years of popularity like the styles in furs. On the whole, *iron* seems to enjoy the most constant favor by practicing physicians. Yet there are many good reasons for past and present misconceptions. Clinical control of patients is very difficult and the number of variables almost infinity, therefore it need not surprise the critic to find that there have been differences of opinion relating to the influence of various drugs on various anemic conditions. Diet factors are only recently coming in for proper study and consequent appreciation.

Our experiments furnish an illustration of the difficulties which arise to perplex the physician who wishes information as to anemia treatment. Our earlier experiments with short anemia periods (15) gave no evidence that iron treatment was of the slightest value. These observations have been confirmed by many other investigators using dogs and other animals (10), (16), (14). The conclusion seemed justifiable that iron was of no value in the treatment of secondary anemia in man.

But subsequent experiments with long standing *severe anemia* give just as positive evidence that iron is of considerable value. These experiments may well emphasize the need of caution in deductions drawn from slightly dissimilar experimental conditions. It is obvious that conditions only slightly different may modify completely a reaction within the body. If we hasten to draw any deductions from these experiments relating to complex anemias associated with marrow disease we are courting trouble. It is to be hoped that the great amount of recent work on experimental anemia will stimulate controlled observations on human anemias (pernicious type, etc.) rather than a flood of careless deductions.

We should not lose sight of the fact that under all our varied experimental conditions in secondary anemia due to hemorrhage, *food factors* (especially various meat products) are most favorable for rapid regenera-

tion of hemoglobin and red cells. It is probable that in human beings *food factors* will be found more efficient in the control of simple anemia than iron or other drugs. Even in complex anemias (human pernicious anemia, anemia with nephritis and cancer cachexia) food factors deserve serious consideration in the clinical management of the blood condition.

REVIEW OF LITERATURE. We cannot attempt a review of the clinical records and opinions relating to drug treatment in anemia. For this task we have not the patience nor would space permit. Musser (10) reports short anemia experiments on dogs in which there was no reaction demonstrable following administration of iron. These experiments were very much like our earlier ones and the results practically identical. Williamson (16) submits a preliminary report on rats and dogs indicating that iron is inert in short anemia experiments and meat feeding quite efficient. Weber (14) comes to similar conclusions but his experiments differ from the above in that the anemia is produced by pyrodin. The destruction of hemoglobin and red cells in the body introduces a factor which will modify the reaction as compared with that following a simple hemorrhage.

There are recorded differences of opinion as to whether iron is absorbed from the intestine. Hall (4) claims that iron is absorbed from the upper intestine, that is, from duodenum and jejunum but not from the ileum. She used mice and claims that the red cells and hemoglobin are increased by iron feeding but does not submit data to establish this claim. Cloetta (3) claims that dogs do not absorb iron from the intestine and submits analyses to show that ingested iron is all excreted. Our experiments given below show clearly that in anemia there may be rapid absorption of inorganic iron.

Germanium dioxide[1] has come in for considerable study very recently and as usual there are wide differences of opinion as to its influence upon the marrow and general blood regeneration. Hammett and Nowrey (5), (6) used the drug in rats and claim that there was considerable increase in red counts. They say there was no evidence of blood destruction. Nowrey (11) later made further observations on white rats and records increase in hemoglobin and red cells during one week of drug injection. He also claims (12) that sodium germanate will cause an experimental polycythemia. Müller and Izzard (9) conclude that there is no evidence that germanium dioxide increases red cell formation by marrow stimulation. Kast, Kroll and Schmitz (7) report favorably upon its reaction under clinical conditions, while Alexander (1) finds no reaction in the few cases observed. Bodansky (2) used dogs and rabbits rendered anemic by drugs and could report only negative influence of germanium dioxide. Minot and Sampson (8) likewise report that germanium dioxide is inert.

[1] We are indebted to Dr. E. A. Slagle of The Germanium Products Company of Trenton, N. J., for a supply of this costly chemical product prepared for clinical use.

EXPERIMENTAL OBSERVATIONS. In our earlier short anemia experiments we used a variety of iron preparations commonly prescribed by physicians in anemia treatment. These preparations were all inert under these experimental conditions (15). In the tables given below we observe a favorable reaction to Blaud's pills given each day. As this simple form of inorganic iron is so potent it did not seem necessary to test out the other familiar iron preparations—colloidal iron, etc.

Table 31 shows a favorable reaction to iron feeding. The contrast with bile feeding is very striking. During two 2-week periods on Blaud's pills this dog averaged about 11 grams of hemoglobin each week, including a "carry over" at the end of the period of about 10 grams. The liver diet period a few weeks later shows a much greater hemoglobin production—a total of 60 grams during two weeks including the "carry over" into the subsequent bread control period.

Table 32 shows a maximal reaction to iron feeding. During two weeks there was removed 43.6 grams of hemoglobin and one week later 18.3 grams which was a "carry over" into the control diet period. These 18 grams should have been removed during the first week's bread period—note the hemoglobin level of 63 per cent. A subsequent liver feeding period gives practically the same hemoglobin production. The contrast with arsenic treatment (sodium cacodylate) is striking and here we observe zero hemoglobin production. This dog gave a remarkable reaction to beef kidney feeding with over 90 grams hemoglobin production. There is a small initial "carry over" in the very first week of the table due to the preceding kennel diet.

Table 33 shows a number of characteristic reactions to Blaud's pills. In these experiments the reaction to iron is slightly more favorable than to beef muscle feeding but we must recall again that in both meat feeding experiments there was a considerable gain in body weight. Under these conditions there is an increase in red cell volume and probably a deviation of substances in the food intake for growth purposes which otherwise would go to form hemoglobin and red cells.

Table 34 gives the necessary data to show that arsenic is almost inert in these anemia experiments. There are dozens of food factors which are much more potent than any form of arsenic under these conditions. The two experiments with sodium cacodylate are almost negative and one experiment in table 32 quite negative. When Fowler's solution is given there is a slight reaction in dog 21-67 but nothing to excite any enthusiasm. The very low color and hemoglobin indices are of interest. Dog 18-114 gives the most favorable reaction to arsenic but we must recall that this dog is abnormally active in red cell and hemoglobin reaction in control periods. Such animals are uniformly active under various conditions as noted above (table 21) and give maximal reaction to a great variety of food factors (see also table 35—dog 18-114).

TABLE 31

Dog 20-71 bull, male, adult. Experiment begins October 17, 1923

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLEED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 500	100	13.8	924	3.2	0.59	2.38	15.8	38	**0**
Bread (C) 500	89	13.9	972	3.5	0.55	2.50	15.4	39	**0**
Cane sugar 75, dext. 50*		12.2	841	4.7	0.56	2.27	23.4	53	**0**
Cane sugar 75, dext. 50*		11.1	737	4.8	0.56	2.26	23.9	54	**10.7**
Bread (C) 500	68	11.9	817	3.7	0.53	1.95	20.0	39	**12.5**
Bread (C) 500	88	12.1	834	3.5	0.59	2.14	19.3	41	**0**
Pig bile 3 t. br. (C) 500	76	11.9	835	5.1	0.49	2.19	22.6	50	**0**
Pig bile 3 t. br. (C) 500	79	12.3	879	5.1	0.45	1.85	25.0	46	**0**
Blaud's pills 3, br. (C) 500	81	11.7	807	6.0	0.44	1.97	26.7	53	**0**
Blaud's pills 3, br. (C) 500	63	11.6	800	5.1	0.48	2.04	23.9	49	**11.0**
Blaud's pills 3, br. (C) 500	74	11.9	850	3.8	0.72	2.03	27.1	55	**0**
Blaud's pills 3, br. (C) 500	56	11.6	814	3.9	0.56	1.97	22.0	43	**24.6**
Bread (C) 500	56	11.4	732	5.3	0.47	2.11	23.7	50	**0**
Bread (C) 500	55	11.1	683	4.6	0.47	1.96	21.9	43	**13.5**
Beef 300, br. (C) 300	100	13.6	892	4.5	0.57	2.19	23.4	51	**0**
Beef 300, br. (C) 300	100	15.0	984	4.1	0.56	2.15	21.3	46	**0**
Beef 300, br. (C) 300	100	16.0	1030	4.6	0.55	2.31	22.1	51	**0**
Bread (C) 500	100	15.5	966	5.9	0.44	2.14	16.0	34	**8.8**
Bread (C) 500	63	15.1	839	4.2	0.50	1.94	21.6	42	**0**
Liver (beef) 200, br. (C) 300	100		900	4.4	0.55	2.22	21.8	48	**0**
Liver (beef) 200, br. (C) 300	100	16.5	784	6.1	0.59	2.40	30.3	72	**21.3**
Bread (C) 500	98	16.0	854	4.0	0.64	2.44	21.0	51	**31.9**
Bread (C) 500	98	15.7	766	4.2	0.59	2.50	19.9	50	**10.9**
Egg alb. 150, br. (C) 350	100	15.9	782	4.4	0.58	2.42	17.5	42	**16.6**
Egg alb. 150, br. (C) 350	100	16.0	790	4.5	0.58	2.46	21.4	53	**15.6**
Bread (C) 500	96	16.1	758	4.4	0.60	2.29	17.7	41	**13.3**
Bread (C) 500	85	16.1	776	4.6	0.51	2.70	22.9	62	**11.8**
Gelatin 50, br. (C) 400	96	15.8	769	3.3	0.67	2.38	18.5	44	**11.6**
Gelatin 50, br. (C) 400	99	16.1	724	6.0	0.58	2.73	23.0	63	**23.9**
Bread (C) 500	74	15.7	690	3.6	0.61	2.40	18.2	44	**13.6**
Bread (C) 500	69	15.6	717	5.8	0.44	2.66	19.4	52	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin}}{\text{Red cell hematocrit per cent}}.$$

* Cane sugar 75 grams dextrose 50 grams dissolved in 250 cc. water and given daily by stomach tube.

TABLE 32

Dog 21-64 bull, female, adult. Experiment begins October 17, 1923

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLEED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 450	46	14.1	818	3.4	0.59	2.36	17.0	40	**14.0**
Bread (C) 450	64	14.5	966	3.7	0.49	2.38	15.2	36	**0**
Liver (beef) 450	100	14.5	866	5.2	0.77	2.22	35.9	80	**0**
Liver (beef) 450	100	14.0	747	5.6	0.86	2.24	43.3	97	**47.3**
Bread (C) 450	67	14.5	906	3.8	0.78	2.34	25.2	59	**26.6**
Bread (C) 450	100	14.8	894	3.9	0.70	2.38	23.0	55	**0**
Blaud's pills 3, br. (C) 450	89	14.7	826	4.7	0.85	2.26	35.3	80	**0**
Blaud's pills 3, br. (C) 450	82	14.4	960	3.8	0.63	1.93	24.6	47	**43.6**
Bread (C) 450	85	14.2	959	4.7	0.67	2.08	30.1	63	**0**
Bread (C) 450	49	13.6	934	3.7	0.59	1.88	23.3	44	**18.3**
Chloroform, br. (C) 450*	44	12.6	813	5.0	0.48	2.05	23.4	48	**0**
Chloroform, br. (C) 450	55	12.7	816	4.4	0.49	2.10	20.6	43	**0**
Liver (beef) 400, br. (C) 300	96	14.8	952	5.2	0.59	1.98	23.4	46	**20.6**
Liver (beef) 400, br. (C) 300	100	15.5	1000	4.7	0.70	2.11	21.1	45	**28.3**
Bread (C) 450	90	15.1	956	4.3	0.66	2.25	25.1	57	**0**
Bread (C) 450	100	15.0	1000	4.3	0.69	2.19	19.5	43	**30.9**
Arsenic, br. (C) 450†	87	15.0	1017	3.2	0.70	2.19	20.5	45	**0**
Arsenic, br. (C) 450	88	14.8	967	4.4	0.52	2.07	22.2	46	**0**
Kidney (beef) 400, br. (C) 300	100	17.0	972			2.19	23.5	51	**26.0**
Kidney (beef) 400, br. (C) 300	100	17.2	849	5.9	0.71	2.31	24.3	56	**35.1**
Bread (C) 450	69	16.1	892	4.4	0.68	2.48	24.2	60	**17.5**
Bread (C) 450	87	16.3	866	3.9	0.74	2.46	23.6	58	**15.5**
Egg yolk 85, br. (C) 350	100	16.4	850	4.8	0.72	2.48	27.8	69	**19.0**
Egg yolk 85, br. (C) 350	100	16.6	818	4.8	0.57	2.48	18.9	47	**44.4**
Bread (C) 450	100	16.7	902	4.8	0.67	2.59	21.8	56	**23.1**
Bread (C) 450	72	16.0	888	4.2	0.68	2.02	19.6	40	**15.5**
Egg alb. 150, br. (C) 350	100	16.7	980	4.0	0.56	2.38	18.7	45	**0**
Egg alb. 150, br. (C) 350	100	16.6	872	5.0	0.56	2.36	21.6	51	**16.8**

TABLE 32—*Concluded*

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Bread (C) 450	99	16.7	846	4.6	0.51	2.22	17.0	38	**19.6**
Bread (C) 450	78	16.7	846	3.8	0.54	2.31	17.7	41	**0**
Gelatin 50, br. (C) 400	77	16.6	885	3.8	0.53	2.22	18.0	40	**0**
Gelatin 50, br. (C) 400	96	17.1	866	4.8	0.46	2.22	19.8	44	**0**
Bread (C) 450	78	16.5	802	3.8	0.65	2.38	20.6	49	**13.6**
Bread (C) 450	90	16.5	768	4.4	0.42	2.48	15.1	37	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Chloroform 2 cc. in olive oil and milk by stomach tube once at beginning of each week.

† Arsenic in form of sodium cacodylate 1 cc. of a 1 per cent solution subcutaneously daily.

Table 35 gives four different experiments with germanium dioxide. Adult human dosage was employed in all cases—two by mouth and two subcutaneously. This means about five times or more the dose per kilo body weight used in human cases. There was no change in the dogs' condition which could be attributed to the drug and we may say that it is practically inert under these simple experimental conditions. We cannot accept without much accurate and favorable data the claim that germanium is a marrow stimulant.

Chart E (dog 20-71) shows in graphic form the fluctuations in plasma volume (upper curve) and the anemia level in terms of hemoglobin per cent (lower curve). The amount of hemoglobin removed in grams is shown by the solid columns. The columns with the cross hatching indicate the grams of hemoglobin removed during control periods. This hemoglobin is to be regarded as a "carry over" from the preceding favorable diet period.

Discussion. The color index and hemoglobin index show fluctuations in these tables which have considerable significance. The hemoglobin index equals hemoglobin per cent divided by red cell hematocrit per cent, and indicates changes in saturation of the red cell stroma with hemoglobin. These indices may be considered together but we shall attempt to review only a few of the important points relating to fluctuations observed in these values. We are learning by means of diet control to modify both color index and hemoglobin index but do not at this time wish to take up these questions in detail.

TABLE 33

Inorganic iron by mouth

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
Dog 24-2 bull, male, young adult									
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 500	90	12.8	883	3.9	0.56	1.87	23.4	44	**0**
Bread (C) 500	88	12.9	843	5.2	0.50	2.00	25.9	52	**0**
Blaud's pills 2, br. (C) 500	62	12.4	886	5.7	0.46	2.10	25.0	52	**11.3**
Blaud's pills 2, br. (C) 500	71	12.3	848	4.8	0.58	2.05	22.3	46	**30.1**
Bread (C) 500	71	12.3	844	4.8	0.49	1.97	23.7	47	**0**
Bread (C) 500	64	12.2	748			2.00	25.5	51	**0**
Beef muscle 300, br. (C) 300	100	14.0	962	5.7	0.48	2.04	20.6	42	**13.8**
Beef muscle 300, br. (C) 300	100	14.6	990	5.3	0.38	1.99	20.3	40	**0**
Bread (C) 500	100	14.9	990	4.8	0.46	2.00	22.0	44	0
Dog 24-3 bull, male, young adult									
Food, grams per day:									
Bread (C) 500	80	11.9	929	3.6	0.49	1.72	20.5	35	**0**
Bread (C) 500	100	12.4	936	4.0	0.48	1.70	22.8	39	**0**
Blaud's pills 2, br. (C) 500	96	12.8	1000	5.1	0.50	2.06	25.0	51	**0**
Blaud's pills 2, br. (C) 500	71	12.2	900	5.1	0.56	2.05	23.0	47	**23.3**
Bread (C) 500	68	11.9	804	3.6	0.74	1.90	19.1	36	**11.9**
Bread (C) 500	79	11.7	780	4.2	0.52	1.90	23.2	44	**0**
Beef muscle 200, br. (C) 300	100	12.9	918	4.4	0.58	2.10	24.3	51	**0**
Beef muscle 200, br. (C) 300	100	13.8	1150	3.4	0.49	1.79	18.6	33	**10.5**
Bread (C) 500	100	14.1	952	3.7	0.58	2.01	21.5	43	**0**
Dog 20-104 bull, male, adult from table 22									
Food, grams per day:									
Chloroform, bread (C) 350*	35	9.0	581	4.7	0.49	2.07	22.4	46	**0**
Chloroform, bread (C) 350	41	9.2	542	4.6	0.62	2.03	20.4	41	**9.6**
Blaud's pills 2 t. br. (C) 350	65	9.0	538	5.2	0.55	1.97	22.4	44	**11.7**
Blaud's pills 2 t. br. (C) 350	62	8.8	493	5.5	0.62	2.08	25.7	53	**17.0**
Bread (C) 350	45	8.4	480	4.8	0.69	2.22	23.0	51	**16.6**
Bread (C) 350	35	8.0	442	5.2	0.73	2.34	32.4	76	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Chloroform 1 cc. in olive oil and milk by stomach tube once at beginning of each week.

TABLE 34

Diet periods. Fowler's solution and sodium cacodylate

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Dog 21-67 bull, male adult									
Food, grams per day:									
Calc. Lact. 4.5, br. (S) 425**	92	10.5	636	4.7	0.46	1.88	23.0	43	0
Calc. Lact. 4.5, br. (S) 400	98	10.5	608	6.5	0.37	1.77	27.1	48	0
Fowler's sol. 0.2 cc., br. (S) 425*	88	10.7	626	6.6	0.37	1.79	27.7	49	0
Fowler's sol. 0.2 cc., br. (S) 425	98	10.5	646	7.1	0.38	1.76	22.7	40	12.5
Fowler's sol. 0.2 cc., br. (S) 425	88	10.3	606	6.6	0.36	1.88	25.7	48	0
Dog 18-114 bull, female, adult—continues table 21									
Food, grams per day:									
Whole milk 450, br. (S) 450	96	14.5	817	5.0	0.50	1.81	22.4	41	19.7
Whole milk 450, br. (S) 450	80	14.5	782	4.8	0.49	1.88	24.9	47	0
Fowler's sol. 0.2 cc., br. (S) 500*	81	14.4	746	6.3	0.42	1.96	21.1	41	12.8
Fowler's sol. 0.2 cc., br. (S) 500	90	14.6	831	5.4	0.47	2.00	22.7	45	11.0
Fowler's sol. 0.2 cc., br. (S) 500	90	14.6	808	5.4	0.40	1.85	23.6	44	0
Dog 24-49 bull, male, young adult									
Food, grams per day:									
Salt mixt. 15, br. (S) 450††	100	13.7	700	5.9	0.48	1.86	25.0	47	9.7
Salt mixt. 15, br. (S) 450	96	14.4	835	5.2	0.41	1.72	24.8	43	0
Bread (S) 450	84	13.4	785	5.8	0.41	1.79	26.8	48	0
Bread (S) 500, salmon 30	100	13.3	782	6.5	0.36	1.62	29.0	47	0
Sod. cacodylate 0.1, br. (S) 500†	100	13.7	804	6.9	0.38	1.81	24.5	44	11.5
Sod. cacodylate 0.1, br. (S) 500	100	14.1	760	5.8	0.42	1.85	26.6	49	0
Dog 20-103 bull, male, adult									
Food, grams per day:									
Milk 450, cream 100 cc., br. (S) 400	100	12.6	764	5.1	0.39	1.72	23.3	40	0
Milk 450, cream 100 cc., br. (S) 400	84	13.6	907	6.8	0.35	1.84	25.5	47	0
Bread (S) 400, salmon 50	91	13.3	816	6.7	0.33	1.69	26.2	44	0
Sod. cacodylate 0.1, br. (S) 400†	98	13.1	794	6.8	0.36	1.71	28.8	49	0
Sod. cacodylate 0.1, br. (S) 400	100	13.2	865	6.7	0.41	1.79	25.9	46	10.8
Sod. cacodylate 0.1, br. (S) 400	97	13.2	822	6.3	0.34	1.79	24.0	43	0

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Fowler's solution 0.2 cc., daily, given by mouth with a little food.

† Sodium cacodylate daily subcutaneously, 1 cc. of 10 per cent solution.

** Fifty grams salmon added to diet.

†† McCollum and Simmonds with iron omitted.

TABLE 35

Germanium dioxide by mouth and subcutaneously

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED
Dog 18-114 bull, female, adult—continues table 21									
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (S) 500, salmon 50	74	12.0	624	4.3	0.55	2.13	22.4	48	**17.3**
Bread (S) 500, salmon 50	80	12.0	608	4.1	0.60	2.27	21.7	49	**10.4**
Germanium, br. (S) 500*	90					2.24	20.6	46	**0**
Germanium, br. (S) 500	91	12.4	662	4.0	0.48	1.83	20.9	38	**16.6**
Germanium, br. (S) 500	94	13.0	765	4.0	0.57	1.97	23.1	46	**0**
Bread (S) 500, salmon 50	95	13.5	740	5.5	0.51	1.97	28.6	56	**0**
Bread (S) 500, salmon 50	100	13.2	838	6.0	0.43	2.00	26.1	52	**14.2**
Dog 21-67 bull, male, adult									
Food, grams per day:									
Bread (S) 400, salmon 30	100	8.8	476	4.6	0.62	2.03	18.1	37	**11.3**
Bread (S) 400, salmon 30	97	9.1	524	3.7	0.63	2.22	21.1	47	**0**
Germanium, br. (S) 450, salmon 30†	100					2.11	21.6	46	**11.6**
Germanium, br. (S) 450, salmon 30	97	9.5	559	4.0	0.47	1.81	20.9	38	**0**
Germanium, br. (S) 450, salmon 30	96	9.5	576	5.6	0.40	1.90	23.7	45	**0**
Bread (S) 425, salmon 50	86	9.8	574	6.0	0.42	2.00	25.0	50	**0**
Bread (S) 425, salmon 50	95	9.9	622	5.1	0.39	1.91	21.1	40	**11.6**
Dog 23-3 bull, male, young adult									
Food, grams per day:									
Bread (S) 550	96	12.4	625	4.5	0.56	1.96	22.6	44	**7.9**
Bread (S) 550	100	12.8	732	4.7	0.45	1.84	22.9	42	**0**
Germanium br. (S) 550†	100	12.8	690	5.1	0.47	1.99	24.9	48	**0**
Germanium br. (S) 550	100	13.1	686	5.9	0.40	1.81	26.2	47	**0**
Germanium br. (S) 550	100	13.1	705	6.1	0.42	1.95	22.4	44	**9.7**
Bread (S) 550	100	13.3	680	5.6	0.42	1.98	23.7	47	**0**
Dog 24-26 bull, male, young adult									
Food, grams per day:									
Bread (S) 450	100	10.4	534	5.0	0.53	1.97	24.0	47	**9.9**
Bread (S) 450	100	10.6	624	4.1	0.53	1.92	22.8	44	**0**
Germanium, br. (S) 450*	98	10.7	636	4.8	0.51	1.94	25.5	49	**0**
Germanium, br. (S) 450	90	10.8	531	5.6	0.44	2.00	23.4	47	**9.9**
Germanium, br. (S) 450	50	9.7	595	4.4	0.49	2.00	21.4	43	**0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

* Germanium dioxide subcutaneously 10 cc. daily = 1.05 grams in 3 weeks.

† Germanium dioxide by mouth 20 cc. daily = 1.05 grams in 3 weeks.

Solutions prepared for human use and adult dose given to each dog.

The various dogs in our colony before anemia is produced will present normal hemoglobin values of 125 to 150 per cent—(100 per cent hemoglobin = 13.8 grams hemoglobin per 100 cc. of whole blood). Likewise the color indices at this time will average 0.90—variations rarely exceeding 0.80 and 1.00. The hemoglobin indices will average 2.60 to 2.70—variations rarely exceeding 2.50 and 2.90.

As the anemia is produced we note a progressive drop in the color index. In most of the tables the period of bleeding and preliminary diet control is sufficient (1 to 3 weeks) to reduce the color index from 0.90 to 0.60 or 0.70. In table 22 we observe a dog with high color index in which there is a slow progressive drop in the color index during many weeks of the experiment. During this same period there is nothing like a similar

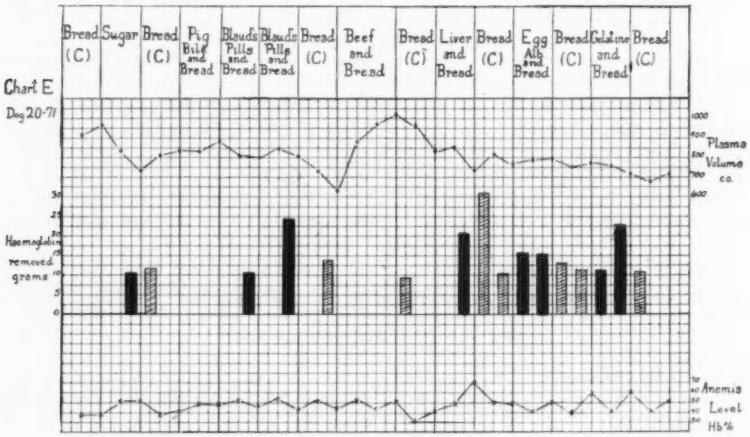

Chart E

change in the hemoglobin index which in all these experiments is a figure which fluctuates relatively little, rarely exceeding a 20 per cent change, in contrast to the color index which frequently shows more than 50 per cent variation.

As the *color index falls* we are prone to think of this change in terms of *pale red cells* which contain progressively less hemoglobin per unit cell volume. The hemoglobin index should show a parallel diminution if the red cells are unchanged but for a decreased hemoglobin content. It is obvious that there are no data here to favor this explanation but rather ample evidence to show that the *red cells become smaller in size* while the content of hemoglobin per unit volume of red cells decreases only slightly if at all.

This indicates that during anemia stress these dogs produce red cells which are a little smaller than normal but contain almost a normal hemoglobin content. The *small size* of the red cell is vastly more concerned with the low color index than is the slight unit decrease in the hemoglobin which is held in the red cells. It is interesting to recall that recent altitude studies in man (13) indicate that under stress of oxygen want at high altitude the new red cells formed are smaller in size than normal but contain the normal amount of hemoglobin.

It is probable that these cells which are slightly smaller than normal may be even more efficient in oxygen exchange and transfer because of the necessary relative increase of red cell surface per unit mass. It is obvious from these figures that the color index is of relatively little value alone without a knowledge of the cell mass as measured by the hematocrit.

It may be argued that the red cell volume may vary due to fluctuations in plasma salt content and other variables. This may be true in condition of shock due to many agencies including hemorrhage and these objections may apply to short period experiments with violent disturbances. We do not accept these objections as applicable to our experiments because of the uniform conditions and bodily health that obtain in all our experimental periods. No hematocrit readings are taken less than 48 hours after a hemorrhage which allows ample time for circulatory adjustment. Under such conditions we believe our red cell hematocrit values mean mass changes in red cell stroma and hemoglobin volumes not merely a swelling or shrinkage of the original stroma and hemoglobin.

SUMMARY

Short anemia experiments by the writers and many others show that iron treatment is without effect upon subsequent hemoglobin and red cell regeneration. The same is probably true for human beings.

Long continued severe anemia due to hemorrhage may be associated with iron depletion and these experiments in dogs show a favorable reaction to iron treatment (Blaud's pills). The same is probably true for human beings.

From these two simple types of experimental anemia we have no data which enable us to predict the reaction to drug treatment to be expected in the complex human anemias, so often associated with chronic infection and intoxication.

Variations in the color and hemoglobin indices are discussed. The low color index in these anemia experiments is referable to *small* red cells rather than *pale* red cells—or the hemoglobin content is almost normal in these red cells which are distinctly subnormal in size.

Germanium dioxide is a drug which under these experimental conditions is almost inert.

Arsenic in the form of cacodylate or Fowler's solution may give a slight reaction or be quite inert as influencing hemoglobin regeneration in these experiments.

Under a variety of experimental conditions in anemia due to hemorrhage the reactions to iron may vary, yet the *favorable reaction to liver feeding is a sustained and uniform maximum.*

Food factors deserve serious study under controlled conditions by physicians who would understand the complex anemias associated with human disease.

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BLOOD REGENERATION IN SEVERE ANEMIA

IV. Green Vegetable Feeding

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Received for publication February 13, 1925

Our short anemia experiments with various green vegetable feeding gave evidence indicating that spinach was somewhat more favorable for hemoglobin and red cell regeneration than other green vegetables, for example—beet tops, Brussel's sprouts, celery and parsley. With the exception of spinach these green vegetables were practically inert in short anemia experiments. A brief review of these experiments appears in a recent paper (3). Recent papers by Scott (1), (2) indicate that in rats the blood regeneration in anemia may be more rapid on bread, milk and spinach than with bread and milk alone.

The severe anemia experiments given below show that the reaction may vary considerably in different dogs at different times. The same dog may react more favorably to spinach and lettuce feeding, and little if any to asparagus. Another dog may regenerate the same amount of hemoglobin and red cells on asparagus feeding as during a spinach period. On the whole spinach and beet tops seem somewhat more potent than lettuce and asparagus feeding in prompting hemoglobin and red cell regeneration. The margin is very small, however, and it may be safest to state that these green vegetables are much alike and only moderately favorable for hemoglobin and red cell regeneration under the conditions of our experiments.

Experimental observations. Tables 41 and 42 give characteristic reactions to green vegetable feeding and the difference is slight when we compare these vegetable periods with control bread periods. It seemed desirable to include in these tables a few other diet periods some of which will be taken up again in subsequent papers.

Table 41 shows the first experiment (dog 20-104) which is the continuation of table 22. One can observe in table 22 the reaction of this dog to a great variety of food factors. We note in table 41 that this dog reacts to spleen feeding much like the subsequent reaction to green vegetables. This particular dog reacts a little more favorably to beet greens than to spinach and least to asparagus. This dog probably regenerates on a bread diet alone about 1 to 3 grams of hemoglobin per week. During the

TABLE 41

Green vegetable feeding

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
Dog 20-104 bull, male, adult—continues table 22									
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Food, grams per day:									
Bread (C) 350	35	9.1	489	4.6	0.50	2.44	18.7	46	**0**
Spleen (calf) 150, br. (C) 250	100	9.9	532	4.0	0.57	2.38	19.1	45	**0**
Spleen (calf) 150, br. (C) 250	100	10.4	531	5.1	0.54	2.40	19.0	46	**11.1**
Bread (C) 350	44	9.9	472	5.6	0.45	2.48	20.2	50	**0**
Bread (C) 350 salmon 50	93	10.2	502	4.8	0.61	2.68	22.0	59	**11.7**
Asparagus 150, br. (C) 250	96	9.8	527	3.8	0.48	2.19	16.5	36	**0**
Asparagus 200 br. (C) 300	63	9.4	538	4.2	0 52	2.22	19.5	43	**0**
Spinach 200, br. (C) 300	71	9.2	504	4.8	0.48	2.19	20.9	46	**0**
Spinach 200, br. (C) 300	84	9.3	540	5.8	0.48	2.19	21.3	47	**14.8**
Bread (C) 350, salmon 25	96	9.8	520	5.9	0.42	2.22	22.4	50	**0**
Beet greens 200, br. (C) 300	64	9.3	532	4.4	0.52	2.25	20.1	45	**12.0**
Beet greens 200, br. (C) 300	56	9.3	528	4.6	0.55	2.22	22.7	50	**10.7**
Dog 20-103 bull, male, adult									
Food, grams per day:									
Egg Alb. 150, br. (C) 350	100	11.9	525	6.0	0.60	2.50	19.5	49	**22.8**
Egg Alb. 150, br. (C) 350	99	12.2	660	4.8	0.77	2.46	15.7	39	**11.9**
Bread (C) 400	61	11.9	658	4.0	0.54	2.27	19.0	43	**0**
Tripe 200, br. (C) 300	100	12.8	662	4.8	0.51	2.44	19.9	49	**0**
Tripe 200, br. (C) 300	94					2.26	19.8	45	**12.7**
Tripe 200, br. (C) 300	79	13.4	742	3.9	0.51	2.22	17.8	40	**0**
Asparagus 200, br. (C) 300	74	12.7	862	5.1	0.46	2.04	17.2	35	**10.3**
Asparagus 200, br. (C) 300	80	11.7	743	4.3	0.51	2.08	21.2	44	**0**
Lettuce 200, br. (C) 300	95	11.6	748	5.2	0.41	2.03	21.1	43	**0**
Lettuce 200, br. (C) 300	97	11.2	746	6.4	0.42	1.82	26.5	48	**12.2**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

first bread period there was little food consumed and we note a blood plasma shrinkage which is a constant reaction in severe anemia.

The second experiment in table 41 (dog 20-103) shows an unusual reaction to egg albumin feeding. The usual reaction is considerably less than this. On the contrary the reaction to tripe (fresh cooked beef stomach) feeding is considerably less than usual. This dog shows similar reactions to both asparagus and lettuce feeding.

Table 42 shows three experiments on different dogs. The first experiment (dog 19-104) is a continuation of table 2 given above and we may refer to that table for the reaction to many other food factors. The first bread period shows a slight "carry over" from a preceding 2 weeks' spleen feeding period. The asparagus period seems quite negative and we find constant weight and hemoglobin level with complete food consumption. There is a little more favorable reaction with spinach and lettuce. The contrast with one week's liver feeding is notable and we observe much regeneration of hemoglobin and red cells. In addition to the hemoglobin removed we note a sharp rise in the hemoglobin level, red count and hematocrit.

The second experiment in table 42 (dog 20-1) is a continuation of table 1 given above. There is a moderately good production of hemoglobin and red cells during both diet periods of spinach and beet greens. There is a little "carry over" into the control bread period following beet greens feeding. This dog gave a very slight reaction to feeding of brains and this is unusual—compare table 1 where this same dog gave a very favorable reaction with a regeneration of 20 to 30 grams of hemoglobin per 2-weeks' feeding period of brains and bread.

The third experiment in table 42 (dog 21-67) illustrates a difference in reaction to the green vegetables showing more regeneration during asparagus and beet green periods than with lettuce feeding.

Discussion. Experiments with green vegetable feeding are of particular interest because of the chlorophyll intake. It is generally believed that chlorophyll is very closely related in chemical structure to hemoglobin and it has been suspected that the pigment nucleus of chlorophyll might be taken over by the body as construction material for hemoglobin. Our experiments give evidence that even during maximum stress and stimulus for hemoglobin production, with large chlorophyll intake the normal body does not take advantage of this abundant chlorophyll as digested in the intestine. It is possible that a different reaction might be observed in herbivora who are better equipped to use vegetable material rather than animal protein. In man however we may believe that the utilization of green vegetable chlorophyll may be as inconspicuous as in the dog under maximal stimulus for hemoglobin production.

It is possible that various salts and iron contained in these green vege-

TABLE 42

Green vegetable feeding

DIET PERIODS 1 WEEK EACH	FOOD CONSUMED	WEIGHT	PLASMA VOLUME	RED BLOOD CELLS	COLOR INDEX	HEMOGLOBIN INDEX	RED BLOOD CELLS HEMATOCRIT	BLOOD HEMOGLOBIN LEVEL	HEMOGLOBIN REMOVED BLED
	per cent	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*gms.*
Dog 19-104 bull, male, adult—continues table 2									
Food, grams per day:									
Bread (C) 350	94	10.8	584	4.6	0.51	2.40	19.7	47	**0**
Bread (C) 350	82	10.5	584	5.5	0.54	2.44	18.7	46	**11.3**
Asparagus 150, br. (C) 250	100	10.3	615	4.3	0.52	2.50	18.0	45	**0**
Asparagus 150, br. (C) 250	100	10.3	710	3.9	0.53	2.14	19.4	42	**0**
Spinach 150, br. (C) 250	96	10.0	598	4.7	0.49	2.13	16.8	36	**9.2**
Spinach 150, br. (C) 250	98	9.8	612	5.2	0.41	2.03	21.3	43	**0**
Lettuce 200, br, (C) 300	85	9.8	610	5.9	0.45	2.20	18.9	42	**11.2**
Lettuce 200, br. (C) 300	62	9.5	604	3.9	0.44	1.99	17.2	34	**0**
Liver (beef) 450	77	9.4	578	4.9	0.58	2.50	26.3	66	**11.9**
Dog 20-1 bull, female, adult—continues table 1									
Food, grams per day:									
Spinach 250, br. (C) 400	67	15.4	890	5.2	0.46	2.03	19.0	39	**14.8**
Spinach 250, br. (C) 400	100	15.4	930	4.3	0.48	1.98	20.8	41	**0**
Beet greens 250, br. (C) 400	100	15.3	927	5.5	0.41	2.00	22.7	45	**0**
Beet greens 250, br. (C) 400	93	15.2	866	5.6	0.48	2.38	22.4	53	**16.8**
Bread (C) 450	85	15.2	878	4.6	0.58	2.26	19.2	43	**13.7**
Brains 300, br. (C) 400	98	15.3	862	4.6	0.46	2.13	20.0	43	**0**
Brains 300, br. (C) 400	100	15.8	916	5.3	0.46	2.09	23.5	49	**0**
Dog 21-67 bull, male, adult									
Food, grams per day:									
Asparagus 200, br. (C) 300	89	10.9	700	4.0	0.53	2.09	20.4	43	**0**
Asparagus 200, br. (C) 300	69	9.9	555	6.4	0.43	2.19	20.4	45	**13.9**
Bread (C) 400, salmon 50	93	10.3	624	4.5	0.52	1.90	18.0	34	**9.0**
Lettuce 200, br. (C) 300)	94	9.8	688	4.3	0.47	2.06	19.9	41	**0**
Lettuce 250, br. (C) 300	79	9.6	686	5.0	0.46	2.11	21.9	46	**0**
Beet greens 200, br. (C) 350	41	9.0	600	5.8	0.46	2.19	22.4	49	**10.2**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

tables may be more concerned with this hemoglobin production than is the chlorophyll nucleus. Experiments with the ash of such vegetables will prove interesting from this point of view. It is interesting to note that many *fruits* may be more potent than these green vegetables in anemia periods.

SUMMARY

Our *short anemia experiments* indicated that fresh or canned or dried spinach was rather a favorable diet factor for the regeneration of hemoglobin and red cells. Similar experiments indicated that many other vegetables were inert under similar conditions—for example carrots, Brussel's sprouts, parsley, celery and beet tops.

Our *severe anemia experiments* show clearly that under such conditions the green vegetables are only moderately favorable food factors for the regeneration of hemoglobin and red cells.

Spinach and beet greens appear to be very slightly more potent than lettuce and asparagus but different dogs react in various degrees to these four green vegetables. It may be safest to rate all four alike and state that the presence of large amounts of these vegetables in the diet modifies but slightly the hemoglobin and red cell construction.

Chlorophyll may be very like hemoglobin in its chemical structure but the normal dog cannot utilize much if any of the chlorophyll nucleus for hemoglobin construction even under maximal stress.

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VITAL FUNCTION STUDIES

I. An Analysis of the Dreyer Standards of Vital Capacity

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Received for publication February 16, 1925

In the course of an extended study of objective methods for the diagnosis of endocrine disorders, various so-called vital function tests have been subject to experimental analysis. Among other generalizations of potential value, the concept of Dreyer (1) of evaluating physical efficiency in terms of certain biometric observations, came under investigation. Peabody (2) has shown that there is a definite correlation between vital, or better, lung capacity and the prognosis of certain cardiac disorders. As endocrine disease often produces striking and characteristic deviations of configuration and physical habitus, it was hoped that the application of the Dreyer principles might give information of value. The following report deals with the results of this investigation. The diagnostic aspects of the study are not treated here, reference to diagnostic groups being incidental and solely for convenience. The Dreyer formulation falls naturally into three topics, namely, the chest circumference, the weight, and the so-called vital capacity. They will be considered seriatim.

A. Chest circumference: The circumference of the chest at the nipple line—beneath the breasts in the female—at mid expiration has been equated with the sitting height or, as Dreyer incorrectly terms it, the trunk length. Since it is desired to define averages in order to give concrete magnitude to normal criteria, the comparison is fairly satisfactory. In making this statement, however, two points should be emphasized. First, while the average is a line of reference representing a mathematical equalizing of a large number of observations, the normal is the individual, the departure of whose performance from the average falls within certain limits determined by reasonable individual variation. Second, individual observations can be interpreted only by comparison with other and complementary measurements. A single measurement is usually meaningless without contextual observations. A chest much above the calculated average may mean a magnificently developed athlete or a grotesquely fat person. By equating actual weight and lung capacity,

the chest comparison based upon sitting height assumes a significant magnitude.

B. Weight: In computing the weight standard, Dreyer uses two criteria, namely, the sitting height and the chest circumference, and uses the mean of the two as the calculated normal.

The relative dependence of the sitting height has been voiced by many authors. Some, notably v. Pirquet, prefer the true trunk height eliminating variation in skull height and cervical vertebrae. The sitting height index, i.e., ratio of sitting to total height is an informative supplementary measurement. Pfitzner (quoted by Bean (3)) tabulates numerous results showing values ranging from 0.518 to 0.531 and progressing inversely with the total height. Bean (3) more recently has recorded his own observations of over two thousand subjects and defines 0.524 as the average for his series. Racial influences are demonstrable in the relation while morbid conditions can determine appreciable variation. In the writer's personal series this magnitude has varied from 0.58 in the achondroplastic dwarf to 0.48 in the prepubertal pituitary giant, and other writers have recorded yet wider variations. It is patent that more than 50 per cent of the weight of the whole body lies in the upper half so that for comparative purposes an estimation of normalcy of weight based upon trunk height relation offers a first approximation to a standard. Such comparisons are made to determine individual variations from the norm and the selection of suitable limits for permissible permutation gives a basis for values of relative significance.

With the chest values, however, the results are far less satisfactory. Dreyer's equations were based, presumably, on individuals of relatively normal configuration. An increase in chest girth presupposes a commensurate increase in all other proportions. The three-dimensional character of the change intensifies the influence and appreciable variations in the chest lead to extrapolations which are absurd in their magnitudes. To illustrate,—a patient 150 cm. tall and weighing 129.5 kilos is 5 per cent below the calculated normal weight if Dreyer's conventional average be used. By sitting height comparison alone she is 179 per cent overweight, a figure more nearly in accord with the facts. Similarly, the thin chest of the emaciated leads to results that would imply that the individual were markedly overweight—a conclusion that is far from the truth. The data of any comparison such as the present are far too numerous for presentation in detail. Averages may be compiled, however, and in the following table are given the details of a group of cases. Variation of chest girth is taken as the defining factor. As is to be anticipated, the deviations of the thin are less than those of the obese. Both in sense and amount, however, they are wholly misleading as a gauge of normalcy, the purpose for which they were designed.

On the other hand, the close agreement between observed and calculated average values offers a possible valuable application for the Dreyer tabulations. In the determination of the basal metabolic rate of the

TABLE 1

Deviation of weight from calculated average and trunk height standards

NUMBER OF SUBJECTS	CHEST DEVIATION	WEIGHT DEVIATION		DIFFERENCE
		Average	Trunk height	
	per cent			
1	−24	+6	−27	33
1	−23	+18	−12	30
1	−21	−24	−24	18
1	−20	+29	+0	29
2	−19	+4	−21	25
1	−18	+14	−15	29
2	−17	+3	−20	23
3	−16	+3	−14	17
7	−15	+6	−18	24
6	−14	+6	−22	28
6	−13	+1	−16	17
8	−12	+3	−15	18
6	+20	−3	+32	35
4	+21	−3	+40	43
4	+22	−15	+37	52
6	+23	−9	+42	51
4	+24	−1	+55	56
1	+26	−19	+33	52
2	+27	−1	+53	54
3	+28	+2	+56	54
3	+29	−2	+64	66
3	+30	−0	+61	61
1	+32	−18	+50	68
5	+33	−19	+51	70
2	+35	−31	+35	66
2	+38	−11	+66	77
1	+39	−25	+55	80
2	+43	−23	+69	92
2	+46	−30	+69	99
1	+49	−25	+88	113
1	+52	−40	+61	101
1	+57	−5	+179	184
1	+66	−38	+107	145

individual, the value observed attains significance only when compared with the normal rate computed by any of the several existing criteria. All of these use the patient's weight directly (Harris-Benedict, Dreyer) or indirectly (Aub-duBois) in determining the normal. When the patient

is in bed and in a condition which precludes movement, this quantity can be secured only by estimation of the crudest order. That the Dreyer generalization offers a possible means of calculating this quantity is obvious.

The condition which prevents the weight determination of the patient will usually preclude a measurement of the sitting height. To secure this a series of measurements was made to compare sitting height as usually taken with the distance in a recumbent position between the perineum and top of the head. To secure this measurement, calipers of the usual design were prepared, the indicating arms, however, being 18 inches long to allow for the body thickness of the very obese. The subjects for the initial comparison were groups of one hundred women students at Simmons College and eighty men at the Massachusetts Institute of Technology.[1] The data are collected in table 2.

TABLE 2

Comparison of sitting height and recumbent perineum-crown length

OBSERVATION	MALE			FEMALE		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Sitting height, cm	96.8	82.6	89.6	95.3	79.1	87.0
Perineum-crown, cm	98.6	83.0	91.4	93.7	78.1	85.9
Conversion factor, cm	−4.6	±0	−1.8	+2.8	±0	+1.1

The point of greatest interest lies in the difference in sign of the correction for the two sexes. Different pelvic configuration and, less significantly, distribution of weight in the buttocks offers the probable explanation. Individual deviation here is shown to reach appreciable magnitudes though the women show a greater uniformity than the men. The extremes recorded bespeak the representative character of the subjects. The standing heights varied from 159.5 to 190.3 in the male, and 147.4 to 179.1 in the female.

Inspection of table 1 shows two apparent lines of demarcation within which the Dreyer average offers a fair method of weight prediction. These are found with chests falling within the limits of −19 per cent and +30 per cent. Above and below these magnitudes the errors assume, in the main, invalidating magnitude. Even within these boundaries individual deviations may assume significant proportions. To meet this difficulty, a substitute for the chest girth was sought both in the circumference of

[1] The writer takes great pleasure in expressing his indebtedness to Miss Diall of Simmons College and Mr. McCarthy of the Massachusetts Institute of Technology for their kindness in making these and other measurements on their respective groups. Their coöperation has been invaluable.

the waist taken at the umbilicus and that of the hips. The former was speedily demonstrated to be unreliable.[2] The hip measurements, however, were found to be more satisfactory. By hip circumference is meant the maximum girth around the buttocks taken without attempt to correlate it to any anatomic landmark. The measurements were made on the group of subjects from whom were derived the perineum-crown length. The significant data are collected in table 3. The characteristic curves defined by the points can be expressed by the following equations:

$$\text{Male} \quad \text{Weight (grams)} = 1.616 \times \sqrt[0.426]{\text{Hips (cm.)}}$$

$$\text{Female} \quad \text{Weight (grams)} = 1.387 \times \sqrt[0.429]{\text{Hips (cm.)}}$$

The general applicability of the equations was tested by a series of individuals of relatively normal configuration and widely divergent sizes. The results are collected in table 4.

TABLE 3

Correlation of weight and hip circumference

MALE			FEMALE		
Number of subjects	Weight average	Hip girth average	Number of subjects	Weight average	Hip girth average
	kilos	*cm.*		*kilos*	*cm.*
			19	47.1	87.2
25	56.5	85.8	52	55.4	93.7
36	64.6	90.5	25	63.6	101.3
15	73.0	95.9	8	74.5	107.7

While many of the individuals given below depart significantly from Greek standards, the calculated weights show a fair measure of concordance with those observed. As individuals, they were chosen at random and include babes but a few days old, children and adults. Points were sought for that lay outside the range of the smoothed curves defining the equations. A larger number of observations would undoubtedly contain individuals whose departure from the curve would be more pronounced. In the main, however, it may be stated that with fairly normal configuration the hip equation offers a larger measure of accuracy in weight prediction than either the trunk or chest criteria.

Many cases present, however, in whom there is great abnormality of shape. With these, the combination of sitting height and hip averages

[2] An unpublished study of this dimension in pregnant women showed a daily variation which made it valueless. That this was to be expected is obvious from the character of the tissues involved.

TABLE 4

Comparison of weights observed and calculated by hip formula

NUMBER	SEX	STANDING HEIGHT	SITTING HEIGHT	INDEX	CHEST	HIPS	WEIGHT	WEIGHT FROM HIPS	DIFFERENCE
		cm.	*cm.*		*cm.*	*cm.*	*kgm.*	*kgm.*	*per cent*
1	M.	177.8	91.0	0.511	103.9	109.6	93.4	99.2	+6
2	M.	176.6	94.8	0.537	97.1	102.1	85.0	84.0	−1
3	M.	193.0	101.7	0.527	91.8	96.5	75.0	73.6	−2
4	M.	190.3	94.8	0.498	87.2	94.4	70.5	69.9	−1
5	M.	187.0	92.0	0.492	80.5	90.0	62.7	62.5	±0
6	M.	167.2	82.8	0.495	75.6	79.8	49.7	47.1	−5
7	M.	125.8	61.0	0.485	59.7	60.3	25.7	24.4	−5
8	M.	122.6	62.3	0.508	55.3	55.9	20.9	20.4	−2
9	M.	113.6	60.3	0.531	55.3	53.3	17.7	18.3	+4
10	M.	>52.5	31.5		32.0	27.5	3.93	3.86	−2
11	M.	>55.0	33.5		35.5	27.0	3.75	3.70	−1
12	M.	>55.5	31.5		33.5	27.0	3.54	3.70	+4
1	F.	162.5	88.2	0.543	105.0	119.0	100.9	95.5	−5
2	F.	153.8	83.4	0.542	91.5	118.0	94.2	93.7	−1
3	F.	178.3	88.6	0.497	106.7	109.2	81.6	78.2	−4
4	F.	141.0	78.5	0.557	58.4	82.0	40.2	40.1	+0
5	F.	147.4	79.1	0.536	64.1	83.0	39.9	41.3	+3
6	F.	147.0	78.0	0.531	64.5	79.0	37.3	36.8	−1
7	F.	>52.5	34.0		34.0	31.5	4.38	4.36	+0
8	F.	>49.5	30.5		31.0	25.5	2.74	2.63	−4
9	F.	>47.0	29.0		29.5	26.5	2.74	2.88	+5

TABLE 5

Comparisons of observed and calculated weights in the abnormal

	NO. 1—MALE	NO. 2—MALE	NO. 3—MALE	NO. 4—FEMALE	NO. 5—FEMALE	NO. 6—FEMALE	NO. 7—FEMALE
Standing height, cm.	142.0	135.6	102.9	160.5	166.5	177.0	188.0
Sitting height, cm.	86.5	72.5	52.0	90.5	86.0	91.8	92.0
Chest, cm	155.0	70.0	55.9	118.5	107.0	91.5	84.0
Hips, cm.	160.0	80.0	57.2	154.0	147.0	119.4	116.8
Weight (obs.), kgm.	161.0	41.8	17.7	135.5	106.2	85.0	79.5
Weight from sitting height.	57.0	32.8	11.6	68.8	58.4	72.0	72.5
Weight from chest	323.7	36.7	19.8	296.1	206.7	118.9	88.2
Weight from hips	241.2	47.4	21.6	174.3	156.2	96.3	91.5
Average sitting height and chest.	190.4	33.8	15.7	182.5	132.6	95.5	80.4
Deviation from observation, per cent.	+18	−19	−11	+35	+25	+12	+1
Average sitting height and hips.	149.1	40.1	16.6	121.6	107.3	84.2	82.0
Deviation from observation, per cent.	−7	−4	−6	−10	+1	−1	+3

offers a seemingly closer prediction than the trunk-chest average. A possible exception to this is found in the pituitary case with a girdle obesity that does not extend above the navel. A few cases illustrating the points in question are collected in table 5.

Obviously the method is at best but a first approximation. It may have use, however, in offering a means of weight prediction where the latter is a necessary datum. It is as open to objection as a criterion of normalcy as is the Dreyer formulation.

C. Vital capacity. In computing the lung capacity, conventionally designated vital capacity, Dreyer has used three criteria, namely, weight, chest circumference and sitting height. In addition, he has defined for each comparison three classes based upon condition and occupation. In a recent paper the writer has shown that for the average young man in this country the male "A" standard is most representative, while for the young American woman the female "B" standard is nearer the truth. This latter fact but ill accords with the statement occasionally made that Dreyer's observations were made on groups of war-worn, undernourished and exhausted individuals. In the writer's opinion these class separations defeat the purpose and a single standard should be adopted. Variations from this can then be interpreted with due consideration of vocation, nutritional level or other additional fact. The practise of this laboratory is to use the male "A" and female "B" tabulations as standards and increase the width of the zone of normalcy as the attendant circumstances warrant.

Turning now to the several criteria, one finds in the sitting height the same dependable basis for this comparison as for those already discussed. Again, it should be remembered that the whole principle is that of averages and in the relatively narrow limits of variation of the criterion lies its greatest value. Chest measurements increased by obesity to magnificent proportions lead to absurd extrapolations and in lesser degree the weight criterion becomes more and more misleading as one departs from an average body configuration. The chest criterion is the least happy selection as the accretion of adipose both increases the superficial measurement and lowers the capacity, errors that reinforce each other. It is true that the misleading results of extrapolation are less pronounced in the vital capacity than in the weight comparisons for two reasons.

First, the relative rate of increase of lung capacities is less than that of weight, thus circumscribing the error of extrapolation.

Second, the method of Dreyer which entails averaging the values predicted from three criteria tends to minimize the extremes of variation. Even though the lung capacity predictions are less misleading than those of weight they are still significantly in error if the Dreyer convention be followed.

Before offering concrete illustration of the above, the generalization offered by West (4) may also be discussed. This author establishes two

TABLE 6

Comparison of Dreyer and West standards

NUMBER	CHEST DEVIATION	DREYER			WEST			REMARKS
		Average deviation	Trunk deviation	Difference	Average deviation	Height deviation	Difference	$T'k_D - H't_W$
	per cent	*per cent*	*per cent*		*per cent*	*per cent*		
1	−24	−12	−33	+21	−30	−34	+4	+1
1	−23	+26	+5	+21	+8	+8	±0	−3
1	−21	−10	−31	+21	−12	−15	+3	−16*
1	−20	−3	−14	+11	−17	−16	−1	+2
2	−19	−21	−37	+16	−35	−40	+5	+3
1	−18	−21	−35	+14	−33	−35	+2	±0
2	−17	−3	−19	+16	−18	−21	+3	+2
3	−16	−26	−38	+12	−35	−37	+2	−1
7	−15	+2	−14	+16	−13	−16	+3	+2
6	−14	+1	−14	+15	−15	−18	+3	+4
6	−13	−17	−30	+13	−28	−30	+2	±0
7	−12	−10	−24	+14	−23	−26	+3	+2
6	+20	−29	−13	−16	−21	−17	−4	+4
4	+21	−43	−27	−16	−33	−27	−6	±0
4	+22	−42	−23	−19	−34	−31	−3	+8
6	+23	−44	−25	−19	−33	−28	−5	+3
4	+24	−33	−8	−25	−25	−20	−5	+12
1	+26	−30	−5	−25	−14	−9	−5	+4
2	+27	−27	±0	−27	−17	−11	−6	+11
3	+28	−56	−41	−15	−50	−46	−4	+5
3	+29	−48	−25	−23	−36	−29	−7	+4
3	+30	−46	−23	−23	−34	−23	−11	±0
1	+32	−24	+10	−34	−10	−5	−5	+15
5	+33	−61	−43	−18	−50	−45	−5	+2
2	+35	−40	−12	−28	−20	−15	−5	+3
2	+38	−37	−5	−32	−21	−14	−7	+9
1	+39	−44	−13	−31	−31	−29	−2	+13
2	+43	−64	−40	−24	−48	−41	−7	+1
2	+46	−64	−38	−26	−45	−36	−9	−2
1	+49	−54	−19	−35	−34	−25	−9	+6
1	+52	−38	+9	−47	−12	−4	−8	+13
1	+57	−76	−50	−26	−64	−56	−8	+6
1	+66	−79	−55	−24	−64	−58	−6	+3

* Sitting height index = *0.586*.

criteria, namely, the total height and the area. This latter is a function of the total height and weight so that there is a partial superimposition in the use of the two calculations. Further, in the area comparison ex-

cessive weight makes itself felt unduly but never to the same extent as in the Dreyer tabulation. Simple relationships have been established for men and women. West has also created a third class, that of the athletes, but for reasons given above, it seems to the writer better to limit the prediction to a single class and modify the so-called limits of normalcy in accordance with the nutritional and vocational factors. In West's two criteria there is direct contact with Dreyer's sitting height so that it is to be expected that correlations should be fairly good. This, after all, offers a sound basis for the establishment of a standard. Adopting the convention followed in the earlier tables, the observations are grouped with the chest variation as the index of difference.

TABLE 7

	DREYER		WEST	
	Average	Trunk	Average	Height
Thin	−8	−22	−21	−23
Fat	−45	−23	−33	−27

TABLE 8

Vital capacity deviations in cases of potential endocrine disease

DIAGNOSIS	NUMBER OF CASES	DEVIATION FROM NORMAL
		per cent
Thyroid	64	−28
Gonad	86	−23
Miscellaneous	95	−23
Not endocrine*	125	−18
Pituitary	130	−17

* Cases showing the clinical stigmata of endocrine disturbance but demonstrating on analysis, a non-endocrine etiology.

For reasons given above, the differences here are not so striking as in the weight comparisons. Further, the diminution of the lung capacity has an inferior limit which fact tends to bring all values together by the very nature of the mathematical relationships. It will be obvious, however, from an inspection of the tables that the Dreyer values based upon sitting height and those based on the standing height of the West formulation exhibit the closest correlation and at the same time offer the most representative values.

As an index of impairment of lung capacity it is the practise of this laboratory to take the average of the Dreyer sitting height prediction and the mean of the two West standards. By this means some allowance is made for the weight factor while yet emphasizing the linear dimensions. The relationships can best be shown by averaging the data of table 6, as shown in table 7.

Following the convention indicated above, the comparisons have been applied to a series of 500 cases referred for potential endocrine disease. The results may be briefly summarized in table 8.

While the figures given above would seem to show a relationship between endocrine focus and magnitude of failure, the individual variations observed in each group robs the vital capacity measurements, when considered alone, of any real diagnostic significance. The averages, at best, represent tendencies only.

CONCLUSIONS

1. The Dreyer concept of biometric observations as criteria of health is analyzed.

2. The influence of the chest standard in weight computations is found to be excessive, its use giving possibly misleading results.

3. A relation is established between sitting heights and length from perineum to crown in a recumbent position. Sex determines the sign of the difference.

4. Measurements have been made of the relation of weight to hip circumference and equations derived for the relationship in both sexes.

5. Observations are recorded demonstrating that the hip equation offers a more accurate method of weight prediction in normal individuals than either sitting height or chest circumference.

6. In individuals of markedly abnormal configuration, the sitting height-hip average is more accurate than the "trunk-chest" of Dreyer. A possible exception may be found in the so-called girdle obesities of pituitary disease.

7. The standards for lung capacity are compared and that referred to sitting height is found to be the most indicative. The chest influence becomes unduly large in the very fat and thin and the weight influence is only less misleading. The West standards are found to be more uniformly dependable and an average of Dreyer's sitting height with the mean of the two West criteria is recommended. It is suggested that Dreyer's three classes be replaced by a single standard ("A" for men and "B" for women) and that the limits of allowable variation be fixed by the person's habits and vocation.

8. Deviations from normal standards are observed in various types of endocrine diseases but their magnitudes define tendencies rather than established differences.

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A STUDY OF THE PULMONARY CIRCULATION BY THE TRANS-ILLUMINATION METHOD

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Received for publication February 21, 1925

The great advances in our knowledge of the peripheral circulation made through the direct microscopic observations of the smaller vessels and capillaries, suggested that the pulmonary circulation might be studied in a somewhat similar manner. By such a method ocular evidence should be obtainable as to the changes induced by nerve stimulation, by drugs, or by mechanical influences which affect the pulmonary circulation.

Preliminary experiments soon showed, however, that difficulties not incurred in investigating the flow of peripheral organs arose in such a study of the pulmonary circulation. In the first place, the lungs must be exposed for observation and artificial respiration must be instituted. The respiratory movements of the lungs and to a lesser degree those imparted by the beating heart obviously interfere with microscopic observation of any sort. Secondly, unless special precautions are taken the exposed lungs have a tendency to become congested and to undergo drying, both of which produce conditions in the pulmonary circulation that can not be regarded as quite normal. Finally, it was soon found that the pulmonary vessels are not rendered visible when the technique applicable to other tissues and organs is followed. The earlier experiments consisted in attempting to apply the method first described by Lombard (1) and subsequently employed with such great success by Danzer and Hooker (2) and others in the study of the skin vessels. As is well known, this method consists in rendering the skin relatively transparent by means of a drop of glycerine or oil, illuminating the area so treated with a strong light and examining it by the aid of a microscope of low magnification. It was soon found, however, that oil or glycerine similarly applied to the surface of a lung does not render it transparent so that the vessels can be examined by reflected light. This is probably due to differences in the histological arrangements and in the refractive indices of the cells composing the visceral pleura; possibly also, to the absorption of light by the large amount of blood in the lungs.

The principle of reflected illumination was therefore abandoned and as

a result of numerous experiments the trans-illumination method of studying the pulmonary vascular system was devised. The principle of this method consists in opening the chest, elevating and fixing a quiet lobe of one lung, illuminating an area with a suitable optical system and then examining it by the aid of a low power compound microscope.

METHOD OF LUNG INFLATION. One of the first problems was to devise some method of obtaining a fixed lobe of lung tissue without incurring any impairment of the circulation or the gas interchange within the lobe under observation. To tie off the bronchus of a single lobe for observation, leaving the rest of the lung tissue to perform the respiratory function for the animal proved impracticable, for the blood vessels going to and from the lobe are so inseparably related to the bronchial structures, and any nerve fibers to the ultimate vessels would most certainly be included in the ligature applied to tie off the bronchus.

Naturally, the insufflation method of Meltzer (3) suggested itself as a probable means of avoiding the changes in shape and position of the lung and this method was in fact successfully employed by introducing some modifications. It was originally hoped that a permanent state of lung inflation might be maintained but it was soon found that three to four minutes was the maximum period that such a state of insufflation could be continued without the supervention of dyspnea and some signs of asphyxiation on the part of the animal. Even shorter periods of continued insufflation produced congestive changes as seen under the microscope. Consequently, the final arrangement consisted in allowing the lungs to deflate for exactly every four seconds out of sixty. The continuous inflation for fifty-six seconds proved adequate for making observations provided the inflating mechanism was so nicely regulated that after every deflation the portion of the lung under observation returned exactly to its previous position and thus automatically came into focus again. Inasmuch as this is of considerable importance, the details of the insufflation method as adapted to the condition of these experiments are briefly given.

The air was automatically shut off at periodic intervals by means of a three-way valve actuated by a rotating kymograph and placed in the compressed air line. As shown in figure 1, this valve was designed to produce a gradual and constant opening of the tube connecting with the intra-tracheal tube and at the same time avoid alterations in the pressure of the supplying tube. This was accomplished by the double piston arrangement which operated *a*, to close and open an outlet tube to the same extent that it closed or opened the tube leading air to the trachea; and *b*, after closure of the tracheal connecting tube, allowed air to escape to the exterior at the same rate as it had passed through the tracheal tube, in this way maintaining the supplying pressure constant.

This valve was mounted on the base of a Harvard kymograph and was operated by attaching a cam plate to the rotating rod. The speed of the kymograph and the shape of the cam were so adjusted that during every rotation, which occurred once per minute, the cam pushed the plunger in and out in exactly four seconds.

Inasmuch as some difficulty was experienced in maintaining the inflow tube inserted into the trachea in an exact position during observations and as such shifting tended to modify the precise degree of lung inflation, it was found necessary to construct a special insufflation cannula which contributed greatly to the constant degree of lung inflation. This insufflation cannula consisted of an outer brass tube (e.g., 8 mm. diameter and 6.5 cm. long for medium-sized cats) and an inner brass tube filling exactly two-thirds of the lumen of the outer tube. The inner tube was brought out through the side of the outer tube and connected with the shut-off valve and compressed air system. The instrument was inserted as an ordinary tracheal cannula except that the long end was pushed down until it reached a point just above the tracheal bifurcation. The air under pressure thus entered the inner tube, ventilated the lungs according to the Meltzer principle and returned through the larger tube and trachea communicating above with the larynx, pharynx and nose of the animal.

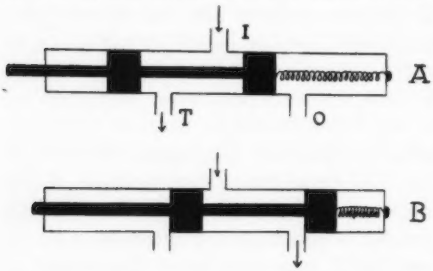

Fig. 1. Diagram of a shut-off valve for producing constant degree of lung insufflation. *I*, air inlet; *T*, tracheal communication; *O*, external outlet. *A* shows position of piston for lung inflation; *B*, position when tracheal communication is shut off.

THE TRANS-ILLUMINATION SYSTEM. The apparatus for trans-illuminating a stationary, lobe of lung consists of *a*, an illuminating system; *b*, a mechanical stage capable of supporting and adjusting the relation of the animal to the projection system, and *c*, a low-power microscope for observation.

The illuminating system (fig. 2 *A*) consists of a 400 watt stereopticon lamp, *1*, and a reflecting mirror, *2*, enclosed in a light-proof, ventilated housing, *3*. A liter Florence flask, *4*, filled with distilled water in which a few crystals of copper sulphate have been dissolved, serves as a condensing lens and a heat absorbing chamber. In order to protect the eyes of the observer and also to limit the illumination to small sections of the exposed lung, a metal funnel, *5*, terminating in a tube about 2.5 cm. wide, is placed between the illuminating system and the animal.

The animal is placed on a mechanical stage shown in cross-section at *B* and in a perspective sketch inserted at *D*. This enables the experimentor to bring various illuminated areas of lung into the field of the microscopic objective by shifting the entire animal in horizontal and vertical directions. This mechanical stage consists of an upper board containing an electrical resistance heating unit, hinged to a lower board. Vertical adjustment of the animal is attained by raising or lowering the upper board upon which the animal lies by means of a cam; horizontal adjustment, by sliding the upper board in metal grooves through a screw thread operated by a handle. After the animal has been fastened to the board, a lobe of lung is carefully lifted from the chest and clipped at the very margin by small clamps, movable on a flexible rod of lead, the two

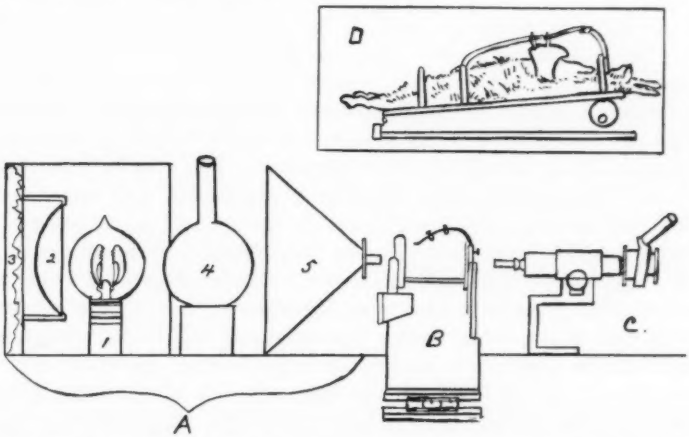

Fig. 2. Diagram of trans-illumination apparatus. *A*, illuminating unit; *B*, end view of mechanical stage; *C*, microscope; *D*, side view of mechanical stage not showing mechanism of horizontal movement.

ends of which are mounted on the upper adjustable board (cf. fig. 2 *D*). In making this adjustment of the lung, extreme care must be exercised not to exert any undue traction upon it nor so to displace the lobe under observation that the blood vessels at the roots are abnormally stretched or kinked. Failure to adopt this precaution readily causes an entire stoppage of the blood-flow or produces a serious congestion. The fixation of the lobe in a quiet phase of respiration and in such a manner that large areas may readily be examined by means of a microscope constitutes in fact the only technical difficulty to be overcome by practice.

The lung tissues not under observation are protected from drying by the use of moist pads of gauze or cotton. The areas under observation are kept moist and warm by allowing a slow stream of warm saline to

flow over them by means of a miniature sprinkling tube mounted above the lung. This saline solution is conveniently kept at an appropriate temperature by placing the flask from which fluid is siphoned above the lamp housing at a height easily determined by trial. It may furthermore be mentioned that the air used for inflation is warmed and partly saturated with moisture by allowing it to pass over a layer of water in a heated chamber.

With the animal thus arranged, a horizontally directed microscope of low power is placed in position as shown at *C* in figure 2. Upon examining the lung in this way it is still found, however, that slight movements are present owing to a cardiac impact. While much can be done by proper placement of the lobe and its separation from the heart by means of cotton pads it is not possible to eliminate entirely the slight cardiac tugs transmitted apparently by way of the pulmonary vessels themselves. While this movement is not serious enough to interfere with the ocular inspection of the lung vessels it prevents photography of the field under observation. In fact, up to the present time photographic records have not proven possible. For this reason it is desirable that all observations be checked by a second competent observer. This can be done through the use of a demonstration eyepiece which enables two experimenters to make observations of any circulatory change at the same time.

Preparation of animal. In the preliminary experiments, cats were used exclusively but later rabbits were also employed. In the latter animals the lungs are in fact more transparent than in the cat and in this respect make really better preparations. As it was desirable to avoid volatile anesthetics during the experiments chloretone and urethane were used at first. In the later experiments, the animals were briefly anesthetized and then pithed, the technic suggested by C. Tigerstedt (4) being used in the case of rabbits. Pithing is in fact essential in observations where the effects of hypercapnia and anoxemia come into play as the resumption of respiratory movements on the part of the animal periodically throws the areas out of focus. It is because rabbits do not seem to recover so well from the shock of pithing that the best observations were made in the case of cats. The lungs were exposed and the ribs cut away liberally on the sides so as to permit lung trans-illumination without much traction on them. Both vagi were exposed in the neck and a cannula placed in the femoral vein for injection of drugs.

Orientation and nomenclature. With the apparatus described it was found possible to view clearly the margins of the lungs for a distance of 15 to 25 mm. from the edge and occasionally to a greater distance. The field of living lung thus examined by the trans-illumination method presents, however, an entirely different picture from what we have become accustomed to interpret in histological and pathological sections,

hence it is of first importance that the observer become oriented as to what he perceives. It is desirable, therefore, briefly to review the well-known anatomical subdivisions of the pulmonary vessels and their relations to the alveoli, bronchioles and bronchi and then indicate in schematic fashion the relations that the observed vessels bear to the better known anatomical scheme.

According to the studies of Miller (5) the branches of the pulmonary artery follow very closely the subdivision of the bronchi, until they reach the apices of pyramidal shaped lobules, as interlobular arteries. From these vessels intralobular branches enter the apex of each lobule. These rapidly break up into an elaborate network of arterioles and

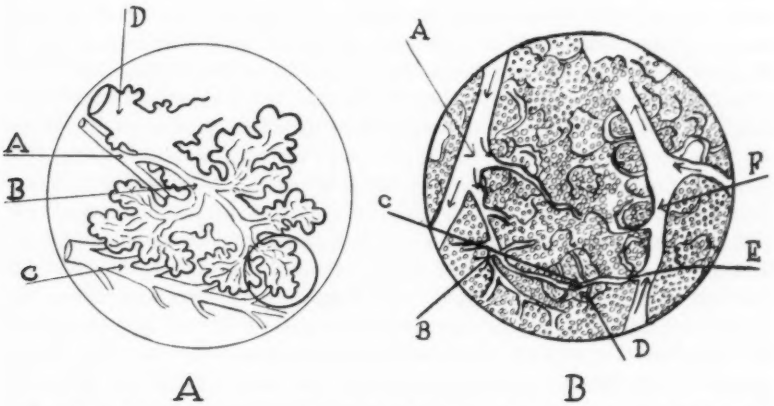

Fig. 3A. Schematic representation of relation of blood vessels to lung lobule. *A*, interlobular artery; *B*, intra-lobular artery; *C*, interlobular vein magnified about 5 times.

B. Drawing of an observed field covering an area approximately equal to that of small circle in *A*—*A*, intra-lobular arteriole; *B*, small arteriole; *C*, capillary; *D*, air cells of an alveolus; *E*, small venule (or capillary?); *F*, intralobular venule.

capillaries which surround and embrace the air cells on all sides. The smaller venules which have their origin in the capillaries of the air sacs, ramify in the connective tissue around the lobules and are arranged in such a manner that the blood flows from the center to the periphery, i.e., into interlobular veins. These interlobular veins in turn merge into larger and larger veins which follow the bronchial system of bifurcation. These relationships are diagrammatically shown in figure 3 *A*. In this drawing an attempt is made to give a probable picture of the structural relationship that would appear if visible when viewed through a hand lens magnifying about 4 or 5 diameters. When an area of the lung is viewed by a compound microscope

with a magnification of about 50, an area equal to about one-tenth of such a field is visible at any one time. In the diagram of figure 3 *A* this would be equivalent to an area indicated by the small circle. It is therefore obvious that a number of different fields have to be studied in order to get a clear picture of the entire circulation in any single lung lobule.

In studying a succession of such fields by the trans-illumination system it is found that the branches of the interlobular arteries and veins and even the larger divisions within the lobule contain so large an amount of muscle and connective tissue in their walls that the flow of blood can not be studied. Consequently, observations of pulmonary blood flow must be restricted to areas lying between these vessels, i.e., the clear fields in which blood flow can be clearly studied consist of the branches that are peripheral to the first intralobular branches. When a field such as is indicated by the small circle in figure 3 *A* is magnified to the size shown in figure 3 *B* the appearance is somewhat as shown in the semi-diagrammatic drawing here presented. It may be stated that this was made from a field actually observed and studied under a magnification of 50 diameters. Such a preparation shows the alveoli and air cells standing out everywhere as bunches of glistening spherules, the refracting surfaces of which change with the degree of lung inflation. When the lungs are relatively deflated the tissue appears denser, the air sacs tend to collapse and the larger vessels between them are not clearly visible. When the lungs are inflated to their normal capacity the appearance is similar to that pictured in the diagram of figure 3. Only here and there is a suggestion given of the characteristics of the polygonal character of the air sacs. Blood vessels of various diameters are clearly seen between the air sacs, the blood within them going not only in every direction of the field but coming out of or receding into the depths of the preparation. For descriptive purposes the larger vessels of the field are designated as large arterioles, *A;* arterioles, *B;* capillaries, *C;* smaller venules, *D;* and venules, *E*. The arterial system is easily recognized by the fact that the blood flows from larger to smaller vessels; the venous system by the fact that the flow is from the smaller to the larger ones. Vessel walls are not distinguishable, the designation of the arteriole, capillary and venous branches being determined rather by the width of the stream bed, the nature of the pulsations and the relative velocities of blood flow.

In the larger arterioles, *A*, in which the walls are sufficiently thinned the blood stream is just visible. The cellular elements appear to be densely packed and move in mass formation. The flow is pulsating with a rapid onward movement during systole and a diastolic decrease in velocity amounting sometimes to a complete stoppage. Expansion of the stream bed during systole and diastole is not observed. In the smaller arterioles, comparable to those labeled *B* in figure 3, the stream is no

longer pulsating, the individual cells are distinguishable and travel through the vessels in ranks of two to six. The smaller arterioles have a great tendency to divide and redivide giving off smaller branches that permit only 1 or 2 cells to pass abreast. These are considered to be capillaries, *C*. In them all pulsation is absent, a continued flow persisting during systole and diastole. The rate of flow varies greatly in different capillaries, however, even in the network surrounding the same alveolus. In nearly every lung, areas can be found where the capillary network around the alveolar cells can be observed. In many fields direct connections between large and smaller arterioles, thence with capillaries and further with smaller venules can be clearly followed as is indicated in the diagram of figure 3. Observations seem to indicate that the network of capillaries for each alveolus is probably derived from more than a single small arteriole. Thus in the vessel shown in the drawing, blood could be distinctly seen flowing from *B* to *E* across and over the top of the inflated air sacs labeled *D*. Two other capillaries crossing this also stood out and in them the blood flowed in opposite direction and at different velocities.

Some difficulty of course exists in differentiating the exact places where capillaries end and veins begin. It would probably be permissible to consider the venous system as beginning with the vessels as they leave the aveoli. In the smallest of such vessels (e.g., *E*, fig. 3) the flow is very similar to that in the capillaries and rarely do the cells pass through two abreast. Indeed it is impossible to say that such vessels are not in fact capillaries. If this is true then the length of the pulmonary capillaries exceeds greatly those in other portions of the body and they empty *laterally* into venules which are comparatively of larger size than in organs supplied by the systemic circuit. In the venules, *F*, the corpuscles are definitely distinguishable and the flow is no longer constant, the onward flow tending to be reduced during systole and increased during diastole. Inasmuch as there is no pulsation in the capillaries and as the forward movement comes during diastole rather than systole this pulsation must be attributed to the backward transmission of pressure changes from the left auricle. This observation is interesting and important in showing how readily and how far back the small variations of pressure in the left auricle can be transmitted into the pulmonary system.

Although many patient observations have been made on a large number of cats and rabbits, no change in the caliber either of the arterioles, capillaries or venules has been seen as long as the degree of lung inflation or heart rate remained constant, nor has any evidence been obtained of the appearance or disappearance of capillary loops around the alveoli. There is therefore no evidence in this research of any active change in the size of the smaller pulmonary vessels.

EFFECT OF CARDIAC SLOWING. The observations so far reported indicate that a continuous flow of blood is maintained through the pulmonary capillaries directly in contact with the air sacs. Inasmuch as these observations were made on cats and rabbits where the heart rate often ranged as high as 240 to 280 per minute and inasmuch as it is well known that the pulmonary arterial pressure falls rapidly and to a very low level when diastole is somewhat longer as in the case of dogs with a slower heart rate, the question naturally arises whether such a continuous flow can be maintained through the pulmonary arterioles when the phase of diastole is much longer. In other words, may we assume that at heart rates normal to man there is sufficient diastolic pressure remaining in the pulmonary arteries to maintain a constant capillary flow?

To investigate this question the vagi nerves were divided and stimulated with weak currents. Unfortunately it was not possible to obtain graded effects, the weakest stimulation producing any effect causing a slowing to 60 per minute or even less. With such sudden changes in heart rate an entirely unanticipated effect on the blood flow in the arterioles, capillaries and venules was immediately obtained on six different animals and demonstrated to other members of the department. With the onset of cardiac slowing the blood flow in capillaries, venules and arterioles comes to a full stop even during systole and during next diastole actually flows backward out of the capillaries and smaller venules into the larger arterioles. This reverse flow during diastole alternating with a systolic stoppage continues for two or three beats, diminishing with each successive beat until a beat occurs where the flow remains stationary. If slowing is continued still longer, a gradual onward movement during systole takes place again with the usual retardations during diastole in the arterioles. The flow in the capillaries first becomes stationary, then reverses, again becomes stationary and then moves onward again. While these observations satisfactorily establish the existence of a constant blood flow through the lung capillaries even when heart rates become rather slow, they bring up for further explanation the curious backflow that occurs during the stage of pressure readjustments. The systolic stoppage of blood can only mean that the pressure in the small venules is equal to that in the small arterioles, while the disastolic backward movement must signify that the pressure is actually higher in the smaller venules. While it is quite possible to postulate pressure changes in the pulmonary artery and veins during the period of readjustment which could account for these changes in flow, it is not at all clear where the backflowing blood is accommodated or how the left heart continues to pump effectively when for several beats the blood flows out of rather than into the venous reservoir.

OBSERVATIONS ON THE INNERVATION OF THE PULMONARY VESSELS. A review of the experimental work relating to the innervation of the pulmonary vessels (Wiggers (6)) leaves the impression that, in spite of many negative reports, the innervation of the pulmonary vessels may no longer be questioned. Such an innervation may conceivably be derived from the sympathetic or from the vagal parasympathetic systems. Most of the experimental work inclines to a sympathetic innervation for mammalian lungs. The positive reactions of the vessels to epinephrin reported by Plumier, Wiggers, Meyer and others as well as the reflex reactions obtained by Schäfer with vagi nerves divided form the chief basis for such an interpretation. The innervation by means of the vagus nerve on the contrary appears to be less clearly demonstrated in mammalian lungs. With the demonstration of such a parasympathetic type of innervation for the lung vessels in the case of the frog and turtle (Krogh, Carlson and Luckhardt) a reinvestigation of this possible source of innervation appeared desirable in the case of mammalian lungs (for references cf. Wiggers (6)).

Evidences of sympathetic innervation. Owing to the complexity of these experiments it has so far not proven feasible to stimulate the sympathetic fibers or ganglia that might conceivably carry vasomotor impulses to the lungs. The observations as to sympathetic innervation were therefore restricted to noting the resultant effects of epinephrin. Unfortunately, as Wiggers (6) has clearly pointed out, the effects of epinephrin on the intact pulmonary circulation are complicated exceedingly by coincident changes on the heart. For this reason nothing short of an absolute reduction in the size of the observed vessels may be taken as definite proof of an epinephrin action.

In spite of the fact that the injection of 1 to 3 cc. of a 1:50,000 solution of epinephrin causes a cardiac acceleration, a definite decrease in the size of the larger and smaller arterioles has been observed in this preparation. At times this was accompanied by a complete cessation of flow in the capillaries, smaller arterioles and venules. Such observations supply further corroboration of a sympathetic control of the smaller vessels.

The effects of the vagus nerves as regards their vasomotor action on the lung vessels. In studying the effects of vagal stimulation it is important to eliminate coincident changes in heart rate, especially as these produce the curious stoppage and reverse flow of the blood already described. Inasmuch as Carlson and Luckhardt have found that the pulmonary vasomotor action in the frog is abolished by atropin but persists after painting the heart with nicotine, a similar procedure was employed in the case of the mammalian heart and found to be satisfactory in preventing changes in heart rate. In addition, a few experiments were performed in which nicotine was given intravenously.

Effect of stimulating the peripheral vagi nerves after previous injection of, or painting of cardiac base with nicotine. Upon stimulation of the peripheral vagi nerves in nicotinized animals where no change in heart rate occurred, a curious effect was generally observed. With the focus unchanged and a constant lung inflation, the vessels appeared to pass out of focus, the alveolar air cells appeared to bulge, to become more globular and to assume a more glistening appearance. On refocusing, the vessels appeared to have sunk deeper into valleys between alveoli. The possibility that these changes may result from stimulation of broncho-motor nerves suggests itself.

This change in appearance during stimulation of the vagi nerves made it difficult to be quite certain in regard to changes in the size of individual vessels but observations on twenty-four different experiments failed to yield any evidence that could safely be interpreted as indicating a change in caliber of any vessel, or an alteration of the blood flow therein. Evidence that the pulmonary vessels also receive a parasympathetic innervation through the vagus was not obtained in this research.

SUMMARY

1. A trans-illumination method for studying the blood-flow in the pulmonary vessels of the cat and rabbit is described. The lungs of a pithed cat are aërated by a modification of Meltzer's method of intratracheal inflation, the constantly inflated lobe is carefully elevated and its margin fixed by clips. If an area toward the margin is trans-illuminated by a suitable optical system, the alveoli with their related blood vessels can be directly observed and studied by means of a horizontally arranged microscope preferably magnifying about 50 diameters.

2. The character of the circulation in the larger and smaller arterioles, capillaries and venules is described. In general, the flow in the larger arterioles is pulsatory, the cellular elements are densely packed while expansion and relaxation of the walls is not observed. In the smaller arterioles, the stream is no longer pulsating, the individual blood cells travelling in ranks of four to six. In the capillaries, the blood corpuscles travel in single file, and in a continuous stream, but the rate of flow varies in the vessels even in the network surrounding the same alveolus. In the smaller venules, the stream is constant and a little more rapid than in the capillaries, individual cells being distinguishable. In the larger venules and the smallest veins, the stream shows a definite pulsation, the onward flow being reduced during systole and increased during diastole, suggesting a back transmission of pressure-changes from the left auricle.

3. Provided that the degree of lung inflation and heart rate remain unaltered, the vessels, i.e., the arterioles, capillaries and venules do not

show any changes in size, nor is there any evidence of disappearance and reappearance of active capillaries.

4. When the heart rate is abruptly slowed the blood flow in the capillaries and smaller arterioles is at first entirely stopped. This is followed by the reverse flow from the venules to arterioles for a few beats, after which the normal onward flow is reëstablished. Continued capillary flow is maintained after this period of adjustment as in the case of more rapid beats.

5. Evidence is presented which indicates that the small vessels of the pulmonary system are affected through the sympathetic nerve fibers but not through the parasympathetic fibers of the vagus. Stimulation of the latter, however, produces changes in the air sacs that can be attributed to bronchomotor nerves.

In conclusion, I wish to acknowledge with thanks the suggestions and criticisms that Doctor Wiggers has given throughout the progress of the work. I am indebted also to Mr. Joseph Dvorak for his conscientious assistance with the technical phases of the experiments and to Mr. W. R. Baker for his aid in the construction of the apparatus.

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EFFECT OF A CALCIUM CARBONATE SUPPLEMENT IN THE DIET OF HENS, ON THE WEIGHT, PROTEIN CONTENT AND CALCIUM CONTENT OF THE WHITE AND YOLK OF THEIR EGGS[1]

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Received for publication February 21, 1925

The studies described herein were made as part of an investigation now in progress at this Station upon the effect of a calcium carbonate supplement in the diet of hens, on the hatchability of their eggs and the production of weakling chicks. In a previous investigation (1) the authors found that the contents, or edible part, of eggs laid in the pullet year, or first laying year, by young hens fed corn and buttermilk, with calcium carbonate, weighed distinctly more than the contents of eggs laid by similar hens, on the same diet, to which the supplement of calcium carbonate had not been added. The present work was undertaken to determine how such increase in weight is distributed between white and yolk, and whether the protein content and the calcium co ntent of white or yolk are affected.

The conditions under which the hens were kept, described in another paper (2), may be restated briefly here. Three lots of 10 white Leghorn hens each were used. All the hens were of the same age, 19 months, and their egg records in the pullet year ranged from 153 to 170 eggs. They were grouped so as to make the lots as nearly equal as possible in weight, vigor and egg-production. The experiment began November 1, 1923, when the three lots were given identical houses, lot 1 being allowed a fair-sized grass range, and the other two lots being confined to their houses during the experiment. Trap-nest records were kept throughout the experiment, and the eggs were weighed.

The diet. All lots were given buttermilk *ad libitum*, with a grain mixture of wheat and yellow corn, and all received some green food, such as cabbage, kale or lettuce, twice each week. Oat straw litter, changed frequently, was used on the floors. Each pen received, each month, an allowance of 20 grams per hen, of a siliceous gravel which had been freed from acid-soluble calcium compounds by treatment with hydrochloric acid and washing.

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The calcium carbonate supplement. From November 1, 1923, to May 1, 1924, lots 1 and 2 were given crushed oystershell *ad libitum*, as a source of calcium carbonate; no calcium supplement was given lot 3 during this period. Beginning May 1, however, oystershell was given to lot 3, and

TABLE 1

Monthly average weights of the total contents, yolk and white, and monthly average egg record for lot 1

	MONTHLY AVERAGE WEIGHT OF TOTAL CONTENTS	MONTHLY AVERAGE WEIGHT OF YOLK	MONTHLY AVERAGE WEIGHT OF WHITE	MONTHLY AVERAGE EGG RECORD
1923	*grams*	*grams*	*grams*	*eggs per hen*
November	54.7	19.4	35.3	8
December	52.0	19.5	32.5	15
1924				
January	53.0	19.8	33.2	11
February	55.7	20.5	35.2	11
March	53.7	18.9	34.8	20
April	52.2	19.4	33.8	22
May	49.7	18.1	31.1	22
June	46.3	17.2	29.1	15
July	48.3	17.3	31.0	18

TABLE 2

Monthly average weights of the total contents, yolk and white, and the monthly average egg record for lot 2

	MONTHLY AVERAGE WEIGHT OF TOTAL CONTENTS	MONTHLY AVERAGE WEIGHT OF YOLK	MONTHLY AVERAGE WEIGHT OF WHITE	MONTHLY AVERAGE EGG RECORD
1923	*grams*	*grams*	*grams*	*eggs per hen*
November	50.5	20.0	30.5	5
December	53.4	19.9	33.5	13
1924				
January	53.7	19.7	34.0	12
February	53.8	19.8	34.0	14
March	53.0	19.6	33.4	17
April	51.0	19.1	31.9	18
May	48.3	19.0	29.3	16
June	45.9	18.1	27.8	5
July	47.5	18.3	29.2	5

withheld from lots 1 and 2. In other respects, all lots were treated alike during the experiment.

Preparing white and yolk. Each alternate egg from each hen was taken for this study, the other eggs having been reserved for the work on hatchability. After weighing, the eggs were broken, the shells together with

the shell membranes were separated, dried 2 hours at 100° and weighed, the difference between the weight of the whole egg and that of the shell and membranes giving the weight of the contents. The yolk was separated

TABLE 3

Monthly average weights of the total contents, yolk and white, and the monthly average egg record for lot 3

	MONTHLY AVERAGE WEIGHT OF TOTAL CONTENTS	MONTHLY AVERAGE WEIGHT OF YOLK	MONTHLY AVERAGE WEIGHT OF WHITE	MONTHLY AVERAGE EGG RECORD
1923	*grams*	*grams*	*grams*	*eggs per hen*
November	49.8	17.8	32.0	5
December	50.7	18.6	32.1	4
1924				
January	51.1	19.4	31.7	4
February	49.2	18.7	30.5	4
March	50.0	18.7	33.3	6
April	50.4	18.3	32.1	8
May	49.9	18.8	31.1	19
June	49.6	18.7	30.9	19
July	49.2	18.5	30.7	15

TABLE 4

Average weights of yolk, white and total contents, 3-month periods

LOT AND TREATMENT	YOLK	WHITE	TOTAL CONTENTS	NUMBER OF EGGS PER HEN PER MONTH	TOTAL WEIGHT OF CONTENTS PER HEN PER MONTH
	grams	*grams*	*grams*		*grams*
Lot 1, Range, Oystershell, average for February, March, April	19.6	34.3	53.9	18	970
Lot 1, Range, No oystershell. Average for May, June, July	17.5	30.4	47.9	18	862
Lot 2. Housed. Oystershell. Average for February, March, April	19.5	33.4	52.9	18	952
Lot 2. Housed. No oystershell. Average for May, June, July	19.1	28.8	47.9	9	431
Lot 3. Housed. No oystershell. Average for February, March, April	18.6	31.3	49.9	6	299
Lot 3. Housed. Oystershell. Average for May, June, July	18.7	30.9	49.6	18	893

carefully from the white, dried on the exterior with absorbent cotton, and weighed. The difference between the weight of the egg contents and the weight of the yolk gave the weight of the white.

The samples analyzed. Composite samples to represent each lot of whites and of yolks, respectively, were made by mixing together all the whites of each kind and all the yolks of each kind, in separate vessels. Nitrogen was determined in the composite samples by the Kjeldahl-Gunning-Arnold method (3), and calcium by the method of McCrudden (1909) (4), after ashing in silica dishes.

Weights of yolk and white. In tables 1, 2 and 3 are given the monthly average weights of the total contents, yolk and white, and the monthly average egg records of lots 1, 2 and 3, respectively, from which have been computed the three-month averages given in table 4.

In table 4 are given the average weights of yolk and white for the 3 months before and after the changes of the calcium carbonate supplement, May 1, together with the average weight of total contents per egg, number of eggs laid per hen, per month, and weight of contents per hen, per month.

The present findings agree with the former observation and show, further, that the increase in weight attending the use of a calcium carbonate supplement falls nearly proportionately upon white and yolk. Though the white accounts for relatively somewhat more of the difference than the yolk, in two comparisons out of three, the difference is not great enough to be conclusive. The most striking difference accompanying the absence of calcium carbonate in the diet is the remarkable diminution in the number of eggs produced per hen, the decrease in the weight of egg contents being relatively a small factor in accounting for the great difference in the amount of egg contents elaborated by the hens. A striking observation is that, although egg production in lot 3 was tripled by the addition of calcium carbonate to the diet, the weight of the egg contents was not increased. Again, although the average number of eggs per hen in lot 1 was not changed by taking away the calcium carbonate supplement, the weight of egg contents decreased about 10 per cent. This may have been because the hens of lot 1 were able to get a partial supply of calcium carbonate from the soil of their range. The diminution in the number of eggs laid in lot 2, confined to the house, was very striking when they were deprived of the calcareous supplement.

Table 5 gives the percentages of protein ($N \times 6.25$) found in the composite samples representing the yolk and white, each month.

The percentages are so uniform that no effect upon the concentration of the egg contents can be traced to the calcareous supplement, or to allowing the hens of lot 1 to range. It is apparent that the total output of protein is in proportion to the output of egg contents, which latter is affected materially by the calcareous supplement, as already shown.

Table 6 shows the proportion of calcium, expressed as calcium oxid, in the yolk and white.

The uniformity in the percentages is very marked. Apparently, the

calcareous supplement does not influence the percentage of calcium in either yolk or white. This accords with our previous experience (5). The percentage of calcium is about twenty times as great in the yolk as in the white.

TABLE 5

Average percentages of protein in the fresh yolk and white, per month

MONTH	YOLK			WHITE		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
1923						
November	16.0	15.7	15.2	10.8	10.6	10.5
December	15.5	15.5	15.6	11.0	11.2	10.4
1924						
January	15.7	15.9	15.1	10.7	10.4	10.5
February	15.6	15.4	15.1	10.2	10.4	10.1
March	15.7	15.7	15.2	10.3	10.8	9.9
April	15.5	15.7	15.5	10.6	9.8	10.3
May	15.5	15.3	15.4	10.2	10.4	10.5
June	15.2	15.9	15.6	10.5	10.1	10.7
July	15.4	15.5	15.3	10.4	10.6	10.2
Average for February, March, April	15.6	15.6	15.3	10.4	10.3	10.1
Average for May, June, July	15.4	15.6	15.4	10.4	10.4	10.5

TABLE 6

Average percentages of calcium oxid (CaO) in the yolk and white, per month

MONTH	YOLK			WHITE		
	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3
1923						
December	0.19	0.15	0.20	0.008	0.008	0.008
1924						
January	0.15	0.16	0.18	0.007	0.007	0.007
February	0.20	0.18	0.17	0.007	0.009	0.009
March	0.17	0.19	0.19	0.009	0.010	0.011
April	0.18	0.17	0.16	0.010	0.008	0.008
May	0.17	0.16	0.17	0.008	0.006	0.010
June	0.19	0.14	0.20	0.009	0.010	0.010
July	0.21	0.19	0.18	0.006	0.009	0.009
Average for February, March, April	0.18	0.18	0.17	0.009	0.009	0.009
Average for May, June, July	0.19	0.16	0.18	0.008	0.008	0.009

SUMMARY

Three pens of 10 White Leghorn hens each were fed a corn-wheat-buttermilk diet, with some green food and siliceous gravel, from November

to May, both with and without calcium carbonate in the form of crushed oystershell. One of the pens was allowed a sod range.

The total contents, yolks and whites of the eggs produced by the hens that received oystershell during the first 6 months weighed distinctly more than those produced by the corresponding hens that had not received calcium carbonate.

When oystershell was added to the diet of the hens that had not received it for 6 months, the weights of total contents, white and yolk, were not increased, but the number of eggs per hen was trebled.

When oystershell was withheld from the diet of the hens that had received it for 6 months, the total contents, yolk and white, weighed less than before.

When oystershell was withheld from the diet of the hens that had received it for 6 months, the number of eggs produced per hen was halved, in the pen confined to the house, but was not affected in the pen that had daily range.

The presence or absence of oystershell in the diet had no appreciable effect upon the percentages of protein and of calcium in the white and yolk.

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SEASONAL VARIATION OF THYROID SIZE IN PIGEONS

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Received for publication February 21, 1925

In studies on the thyroid glands of animals killed in an abattoir Seidell and Fenger (1913), (1914) found that the weights of fresh glands, as well as their iodine content, show a more or less regular seasonal change in cattle and sheep; in the hog the seasonal size changes were considered as different, absent or doubtful. These authors further noted that the data of Martin (1912), (1913) and of Guyer (1913) on English and Scottish sheep also show a similar seasonal variation in both size and iodine content. Herzfeld and Klinger (1922) have stated that data obtained by them on the thyroids of human (Swiss) subjects suggest an enlargement of this gland in winter. They obtained higher percentages of iodine in summer, lower in winter. These authors were apparently wholly unaware of similar observations on seasonal size changes in animals; their data are meager, and by no means cover the various parts of the year. These few series of observations seem to supply all of the information now available on seasonal changes in thyroid size. Even these data are further much complicated by many uncontrolled factors, such as age and diet. It is well known that thyroid size varies notably with the diet; and the diet of the animals used in all the studies mentioned undoubtedly varied considerably with season.

In addition to data specifically based on season, the literature contains some observations on changes of size and histological appearance in the thyroids of animals subjected to periods of heat or cold. In rats kept on a controlled diet and exposed to cold during several days Cramer (1916) observed histological changes which are probably similar to those observable in "winter" thyroids of other animals. Mills (1918) found both directions of change in the thyroids of rabbits, cats and guinea pigs of animals subjected to heat or to cold during one to four weeks. Hart (1922) subjected a group of gray mice to heat, and others to cold, during a few weeks. A reduction of thyroid size was observed in the former group and evidence of increased function in the latter group. When individuals of either group were restored to normal temperatures it is stated that their thyroids soon regained their normal size and histological appearance.

Still another series of studies with more than one point of interest here has been reported by Adler. This investigator (1917) observed that races of the grass frog (*Rana temporaria*) from higher and colder (Alpine) regions have larger thyroids than those from warmer (Adriatic) regions; while those from an intermediate altitude and climate (Bavaria) have thyroids of intermediate size. In experimental cultures of tadpoles he also found atrophic thyroids in animals raised at high temperatures, while hyperplastic thyroids were found in those raised at low temperatures. Adler (1920) further found that, parallel with the increased male-production which results from "over-ripe" eggs of the frog, an increased size of the thyroid is notable in the larvae arising from such eggs; that the thyroid is here differentiated earlier than the gonad; and concluded that the larger and more strongly functioning thyroid acts in a "male-determining direction." He also pointed out that both "over-ripening" and "temperature changes"—the two methods earlier found by Hertwig (1912) to be effective in the modification of the sex ratios in the frog—may both produce their effects through modifications of the thyroid. Adler thus for the first time ascribes to the thyroid an influence upon experimentally obtained sex ratios and upon the determination or reversal of sex development.

Elsewhere Adler (1920b) has contributed much information concerning the relation of the thyroid to hibernation in mammals. He has shown that the thyroids of bats and hedgehogs atrophy in the autumn and again enlarge prior to their awakening in the spring. Apparently he has not noted that his observations on the varying size of the thyroids of frogs from regions of varying temperatures are more or less parallel to those of Seidell and Fenger who reported seasonal changes in non-hibernating mammals. Nor have we found any reference to the fact that the season of atrophy observed in the hibernating mammal is precisely the season when *enlargement* of the thyroid normally occurs in the non-hibernating animal. The closeness of these time relations will, in fact, become clear only after an examination of the data and discussion of this paper.

The data obtained in the present study show that in three kinds of pigeons, kept on the same diet throughout the year, the thyroid notably and promptly enlarges in autumn and winter and becomes smallest in summer; the decrease in size begins promptly with the appearance of warmer external temperature in the early months of spring. It seems probable that the new facts obtained on birds coincide with those already available for mammals much better than appears from a first examination of the matter. These facts, together with the available experimental results on changes due to high and low temperatures, support the following generalization: The thyroids of non-hibernating higher vertebrates normally undergo enlargement in autumn and winter and become smaller

during spring and summer; these size changes are not long delayed but follow promptly, and they are responses effected by the external temperature. That part of the data which was obtained by us in support of this view is, in all probability, essentially free from the complicating effects of diet, care, locality, race, age, length or degree of confinement, health, and body size of the animals examined.

If the above generalization proves to be essentially true it is of considerable significance in biology. It is wholly evident that the seasonal variation of thyroid size here described in pigeons throws important new light upon results earlier obtained by Whitman (1919) and by Riddle (1916) on the seasonal factor in sex and reproduction in pigeons. The present work has been carried out primarily because of its possible bearing on studies on sex and reproduction earlier carried out in this laboratory; these points therefore will be partially considered in this paper.

MATERIAL AND METHOD. In connection with daily laboratory routine continued during three years we have obtained the weights of the thyroids of considerable numbers of pigeons. In this work birds of several species, and of several races or strains within a species, have been killed at all periods throughout the year. The weights of the thyroids were not, however, the sole object of study. Our first purpose was to determine at all periods of the year the size of several of the endocrine glands, and all of the organs of sex and reproduction, in a number of species—each including several inbred races. It is probable that differences in thyroid size exist among the different *races* which constitute each one of the three groups considered here (e.g., common pigeons); it is quite improbable, however, that inequalites arising from this source are responsible for other than minor fluctuations in the data here tabulated for seasonal variation. Individuals of all races were killed during all seasons. The birds killed during the last of the three years differ from those killed in earlier years in being rather more intensely inbred. Nearly all birds had been kept as mated pairs in a single pen; possible results of fighting —with victors and vanquished unequally sharing the food, etc.—have thus been almost completely eliminated.

For only three groups or kinds of birds do we have data sufficient to throw much light on the present topic. Our "common pigeons"—though all were being inbred—contain mixtures of homer, pouter, carrier, Jacobin; also other mongrel strains; and, into some of these races, the wild rock pigeon (*C. livia*) had been but recently introduced and constituted one-fourth to one thirty-second of the ancestry. They are therefore essentially similar to the "pigeons" widely used as a laboratory animal. The "ring doves" are races formed from the blond or cage ring dove (*Streptopelia risoria*) and the later generations of its crosses with the white ring dove (*St. alba*). The so-called "generic hybrids" used here are these last-

named blond-white hybrids into which there has been bred less than one eighth (usually less than one sixty-fourth) of another genus (*Turtur orientalis*).

The birds used were essentially domesticated forms kept throughout life in confinement, but in a degree of confinement found usually to be compatible with reproduction throughout the year. In general, however, a more or less marked retardation of reproduction regularly occurs during autumn and winter. All birds had been kept within (chiefly) glass enclosures from late autumn to early spring; and considerable artificial heat was supplied from November to March to all birds except common pigeons. This latter group—kept in separate buildings—received a smaller amount of artificial heat and this also during a somewhat shorter period. During a few nights of each winter a small amount of ice forms in one or another part of the buildings occupied by common pigeons. All the birds used certainly met lower temperatures during the autumn and winter than during other periods of the year; and this temperature difference was accentuated considerably in the case of the common pigeons.

A mixed grain diet, kept quite uniform—with the rarest exceptions—during many years, was supplied in moderate excess to all birds. The grain mixture given the common pigeons was composed of wheat, cracked corn, kaffir corn and hemp. All other birds were given these grains to which millet and canary seed were added. It will thus be seen that all birds received one grain (hemp) which is rich in tryptophane. All birds were plentifully supplied with crushed oyster shell, grit, cuttle-fish bone, charcoal and rock salt. This diet—at least the food that was offered—was quite the same during all months of the year, and throughout the three-year period covered by the collection of these data. The only variation in the dietary that is known to have occurred is herewith noted—a variation almost certainly without effect on the present data. At the end of the first two years of this period of study about 160 birds (of the 800 listed in table 1) belonging to the three kinds of pigeons listed here were rather heavily dosed with the powder obtained from lettuce (vitamines A and X). The capsules, each containing 0.4 gram of the powder, were given three times at 10-day intervals to these 160 birds, then aged 3 to 35 months. These birds, like other untreated birds of the collection, were killed during all months of the succeeding (last) year. The rate of egg-laying preceding and followng dosage, and more particularly the laying record of the treated with the untreated control, were compared. The time of maturity of the treated young was compared with the untreated young. No measurable difference was found in either case. It is quite improbable that thyroid size was affected. We have the more definite evidence afforded by our first summary of our

thyroid data which was made at the end of the second year of this work. This summary, certainly uncomplicated by this dietary variation, showed the same or a quite similar seasonal variation to that now found for the three-year period. The water supply was from the same source (a free-flowing spring) throughout this period of study.

Birds were killed by decapitation and in turn each of the two thyroids was rapidly freed from adherent substance (in this latter one or more external parathyroids is usually included) and separately weighed. Nearly 2000 pairs of such thyroids have been preserved, but no adequate histological study has yet been attempted. Only a very few thyroids—from both summer and winter—have been examined, and this point must be considered in a later paper. The thyroids of many birds which had been subjected to various treatments are excluded from the data given here. Those excluded are the following: Birds killed *a*, after administration of ox thyroid or iodine-containing substances—used in the course of other studies; *b*, after serial injections of insulin, pituitrin, adrenalin and other glandular extracts; *c*, after subjection to very close confinement ("inactivity") tests. From the "common pigeons" all individuals of two mutational varieties (ataxics, scragglies) have also been excluded. No weights for thyroids obtained from birds less than 6.0 months old are tabulated, though some data of this sort are available. Birds killed by us and found at autopsy to be diseased are not considered here, excepting only the cases of ascariasis. These latter are separately tabulated.

Our autopsy records enable us to treat data from birds dead of disease quite apart from those that were killed by us; they also often identify the presence of particular diseases in birds either dead or killed by us. Doubtless, however, some birds classified as healthy were in fact affected with disease which we were quite unable to discover. The body weight and precise age at death of every bird was known. The variation in the weight of the thyroids is so great that without these several aids to the classification of our material it is doubtful whether a seasonal variation in thyroid size would be recognizable in our data. We are forced to believe that a very much larger amount of improperly classified data would quite fail to give as accurate a measure of seasonal changes in thyroid size as is obtainable from the 800 usable and properly classified pairs of thyroids here considered.

PRESENTATION OF DATA. Our results are presented in tables 1 to 3 and in figure 1. The data of table 1 make it clear that in the case of each of the kinds of pigeons examined it is necessary to divide the thyroid data according to age, to health or disease, and to season. These numerous divisions of the data—as given in table 1—give us figures representing relatively small numbers of thyroids, but it is certain that the

On table 1, page 469, article by Riddle and Fisher, the footnote (†) applies to all age groups—9.0 to 30 months, inclusive.

TABLE 1

The relation of age, disease and season to thyroid weight in various pigeons

KIND OF PIGEON	AGE GROUPS	HEALTHY					ASCARIDIA				
		Seasonal periods					Seasonal periods				
		Number	First	Second	Third	Fourth	Number	First	Second	Third	Fourth
	months		*tenths of mgm.*					*tenths of mgm.*			
Common pigeons	6.0– 6.9	23	351	344	279	244	13	445	380	333	272
	7.0– 8.9	28	380	445	309	225	13	397	303	394	
	9.0–16.9	56	398	371	256	346	29	552	440	388	438
	17.0–19.9	12	408	669	250	588	10	692	348	744*	559*
	20.0–29.9	11	485*	661	655*	564	14	450	483	410*	339
	30.0–80.0†	5	506	482		377*	7		1030	557	1292
Ring doves	6.0– 6.9	22	160	137	121	122	6	117	183		139
	7.0– 8.9	50	169	144	132	143	18	153	148	129	133
	9.0–16.9	51	145	147	120	118	53	161	162	133	163
	17.0–19.9	19	137	156		99*	15	162	148	152*	130*
	20.0–29.9	10	142	142		162	5	137*	152		103
	30.0–90.0†	9	177	209	145	129	8	181	125*	223*	
Generic hybrids	6.0– 6.9	27	154	93	146	96	4				114
	7.0– 8.9	79	186	120	117	174	22	158	154	179	98
	9.0–16.9	61	169	158	145	138	30	168	244	142	149
	17.0–19.9	19	137	164	97*	206	7	146		137*	123
	20.0–29.9	11	205	195	92	156	8	201	138*	144	174*
	30.0–60.0	26	192	215	152	167	19	153	195	207*	121*

* Weight is for thyroids of one bird only.

† Male thyroids only.

TABLE 2

Thyroid size in birds dead of disease

KIND OF PIGEON	AGE GROUPS	TUBERCULOSIS AND LEUKEMIA		OTHER DISEASE	
		Number	Thyroid	Number	Thyroid
	months		*mgm.*		*mgm.*
Common pigeons	6– 8.9			9	41.5
	9– 16.9	2	41.8	21	33.4
	17– 29.9	1	82.0	4	35.0
	30– 75.0	4	109.6	3	90.4
Ring doves	6– 8.9	4	25.9	2	16.0
	9– 16.9	1	25.1	5	13.0
	17– 29.9	1	18.4	3	30.0
	30–111	12	34.1	2	40.8
Generic hybrids	6– 8.9			6	15.8
	9– 16.9	1	9.8	3	15.0
	17– 29.9	1	28.7	2	19.2
	30– 64.0	3	38.7		

TABLE 3

Details of monthly and seasonal thyroid data from birds of both sexes aged 9.0–16.9 months (and used in constructing polygons of fig. 1)

KIND OF BIRD	MONTH	PERIOD OR SEASON	BODY WEIGHT	NUMBER OF BIRDS	AVERAGE WEIGHT OF THYROIDS FOR	
					Month	Season
			grams		*mgm.*	*mgm.*
Common pigeons	October			9 (5)*	46.3 (58.0)*	
	November	First	352	11 (4)	31.4 (33.1)	42.06 (47.31)*
	December		(348)*	9 (3)	50.8 (45.1)	
	January			6 (4)	46.8 (52.3)	
	February	Second	338	3 (2)	45.5 (46.0)	44.53 (45.75)
	March		(336)	9 (6)	42.7 (41.3)	
	April			11 (7)	30.4 (33.1)	
	May	Third	323	10 (5)	32.5 (30.3)	26.60 (27.83)
	June		(306)	16 (4)	20.3 (15.5)	
	July			7 (1)	26.9 (24.0)	
	August	Fourth	333	6 (2)	41.0 (28.0)	34.42 (33.98)
	September		(328)	3 (1)	38.9 (55.9)	
Ring doves	October			6	17.6	
	November	First	166	8	14.7	15.33
	December			5	13.7	
	January			9	14.2	
	February	Second	162	6	13.2	13.84
	March			10	13.9	

Ring doves	April			4	12.0	
	May	Third	159	8	10.4	11.68
	June			11	12.5	
	July			6	12.6	
	August	Fourth	158	3	15.7	13.43
	September			2	12.5	
Generic hybrids	November			21	16.4	
	December	First	161	14	17.7	16.53
	January			11	15.3	
	February			5	16.3	
	March	Second	163	30	16.5	16.77
	April			10	17.8	
	May			9	14.6	
	June	Third	157	8	12.1	14.37
	July			2	12.4	
	August			5	13.3	
	September	Fourth	157	4	16.4	13.56
	October			5	11.6	

* Numbers in parentheses are for females only (see polygons).

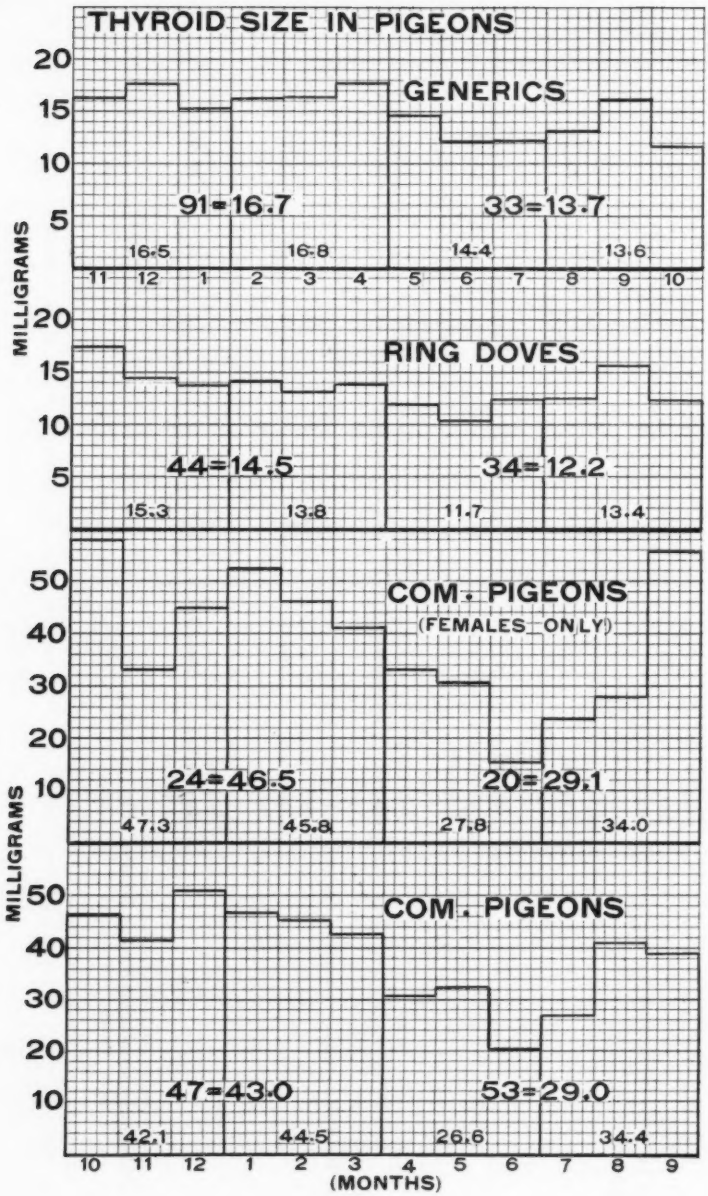
THYROID SIZE IN PIGEONS
GENERICS
91=16.7
33=13.7
16.5
16.8
14.4
13.6
11 12 1 2 3 4 5 6 7 8 9 10
MILLIGRAMS
20
15
10
5
RING DOVES
44=14.5
34=12.2
15.3
13.8
11.7
13.4
COM. PIGEONS
(FEMALES ONLY)
24=46.5
20=29.1
47.3
45.8
27.8
34.0
50
40
30
20
10
COM. PIGEONS
47=43.0
53=29.0
42.1
44.5
26.6
34.4
10 11 12 1 2 3 4 5 6 7 8 9
(MONTHS)

Fig. 1

real facts can be disclosed only through such separation and analysis. Altogether 890 pairs of thyroids—each pair being always considered as one—are tabulated. Of this number 519 are from healthy birds; 281 are from birds affected—often very lightly—with ascariasis; 90 are from birds dead of disease. It is thought unnecessary to publish data from birds killed by us and found with disease other than ascariasis.

Age and thyroid size. Inspection of the data shows that in all of the three kinds of pigeons thyroid size increases with increasing age. That is, a progressive increase occurs after the age of 6 to 7 months, although at that age the birds have attained to approximately their ultimate body size; and most, though not all, are then sexually mature. It is only after 9.0 months that nearly all of the females have attained sexual maturity as shown by the production of eggs. The increment of thyroid size with age is to be seen not only in the healthy birds but also in those affected to one or another degree with ascariasis (table 1). Further, this time relation of thyroid size to age is shown in the data from birds dead of disease (table 2). We shall later refer in some detail to the fact that most females older than 30 months have larger thyroids than have males of this age.

Disease and thyroid size. Birds infested with *Ascaridia* may carry only one or two of these intestinal worms or the intestine may be full of them. These varying degrees of ascariasis may not be expected equally to affect the size of the thyroids of the hosts. A comparison of thyroid size in birds thus affected with thyroids from healthy birds indicates that the thyroids of affected birds are more variable and, usually, larger. These figures, separately given in table 1, clearly show that such thyroids should not be grouped with those from healthy birds. Thyroid weights obtained from birds dead of disease, when compared with those for similar age-groups of healthy individuals, are unexpectedly larger in most cases. This is true despite the fact that a considerable reduction in body weight is usually found in these particular cases. For these two reasons the thyroids of such birds may not be classed with thyroids from normal birds. The same two reasons apply to the thyroids of birds which, though killed by us, were found to be diseased and thus the bearers of thyroids probably at one or another stage between normality and the abnormality clearly observable in birds dead of disease.

Sex and thyroid size. We have examined our data for evidence of sexual size differences. In general, no consistent sex difference is shown by the data now available. In our tabulations the sexes are represented in approximately equal numbers, and the amount of data utilizable for a test of this question is of course only one-half of the amount bearing on the question of seasonal variation. In a larger number of the possible comparisons the thyroids of females seem slightly larger than the male

thyroids, but it is uncertain whether a real difference exists; it is fairly evident that no very considerable sex difference does exist in birds aged less than 30 months. It therefore seems practicable to unite the thyroids of the sexes in an evaluation of seasonal changes in thyroid size.

Reference was made above to the circumstance that the thyroids of females 30 to 90 months old are larger than those of males of similar age. This is observed in common pigeons and in ring doves but not among the generic hybrids, though this may be due to absence of extremely old individuals in the latter group. Male thyroids from healthy birds of the first-named group average 47.0 mgm.; the females 64.2 mgm.; those from males of the second group 16.2 mgm.; the females 24.9 mgm.; those from male "generics" average 19.2 mgm.; the females 17.8 mgm. Birds affected with ascariasis in the three groups give averages which support the differences just noted. For this reason female common pigeons and ring doves aged more than 30 months are not included in the data of table 1. Table 3 and figure 1 supply data for a comparison of male and female thyroids of the common pigeons of one age-group (9 to 16.9 months) at each of the twelve months of the year.

Seasons and thyroid size. Reference may first be made to the data of table 1. The six age-groups, each bearing data for four seasonal periods (here called "first," "second," etc.), involve the separation of the data for each kind of pigeon into twenty-four classes derived from the "healthy" birds and (approximately) another twenty-four classes from those showing ascariasis. Necessarily some of these classes for certain age-groups are composed of very small numbers, and in an evaluation of the data very little importance attaches to weights of a single pair of thyroids; the latter have been specially designated in the table. The tabulation makes it clear that wherever the numbers are more significant, and when healthy birds have supplied the data, practically all age-groups show largest thyroids in "first" and "second" periods and smallest in "third" and "fourth" periods.

Birds affected with *Ascaridia* show the same thing, though in a less consistent and striking way. All age-groups of healthy birds, with one exception (17 to 19.9 months; in which only 10 birds form three groups, none having been killed in the "third" period), show higher average thyroid weight in the six-month period ("first" and "second") of high thyroid weight than in the remaining six months of the year. Thus 17 of 18 tests on healthy birds give a similar result. Further, most of the data obtained from a similar number of groups of birds with ascariasis confirm this result. These results are obtained on all of the three kinds of pigeons. The fact of seasonal change in thyroid size, and also a fair identification of the time or periods involved in these changes, seem to be

On page 474, fifteenth line from top, "30 months" should read "9 months."

clearly established by these data—and by the further analysis of the data given immediately below.

The exact meaning of "first" and "second," "third" and "fourth" seasonal periods should next be made clear. These numerals are used in the tabulations in preference to the names of seasons—fall, winter, spring and summer—to which they respectively more nearly correspond. That correspondence is, however, not complete; and the months involved in each period are not quite the same for the generic hybrids as for the other two kinds of pigeons. In table 1 the data are divided into only four periods. With the data divided into four classes instead of twelve—i.e., corresponding to four parts of the year rather than to twelve months—the resulting numbers in each class are larger and doubtless more accurately express the value of the class. The method used to determine which particular months fall into each of the four classes can be made clearer if the following description is supplemented by reference to figure 1 and parts of table 3.

For the purpose mentioned above the monthly values obtained from the group aged 9 to 16.9 months were taken as a standard in the case of each of the three kinds of birds. The logic and advantages of thus using this particular group are twofold: First, this age-group contained the largest number of birds and should therefore give the most reliable values. Second, practically all of the birds of this group were mature at the lower age limit (9.0 months) of the group; while those at the higher age-limit (16.9 months) were still well removed from the point at which the thyroids of many females tend to become unexpectedly large. The monthly thyroid values of this age-group were next plotted (for each of the three kinds of pigeons) much as now seen in figure 1, except that no vertical division lines were then present. On the monthly values thus plotted the twelve months were so divided as to place the *six consecutive months* with lowest values in one group and the six with highest values in another. The further subdivision of each of the six-month groups into an earlier and later period followed naturally and without choice. For common pigeons and ring doves the first and last months of the six-month period of higher thyroid size were October and March in both cases. For the generic hybrids, however, this period of higher thyroid size began and ended one month later, i.e., in November and April. All data for generic hybrids therefore include November, December, January, as the "first" seasonal period; while this "first" period for all age-groups of other birds is October, November, December (see table 3). The seasonal period which we have called "first" corresponds approximately to the autumn, though this same period for generic hybrids comprises almost as much of winter as of autumn.

On the polygons of figure 1 we have placed the average weights of thyroids of each 6-month and 3-month period. On this figure also it was possible to plot separately the curve or polygon for the *females* of one kind of pigeon. It is important to our later discussion that the values found for the two sexes are shown to apply to females when this sex is considered alone. Our data show that this holds for all of the three kinds of females. The data here separately tabulated for female common pigeons show that *from spring to autumn the size of the female thyroid undergoes a progressive decrease.* This is the precise period found by Whitman and by Riddle to be involved in experimental changes in sex ratios and other reproductive processes; and, from the first, the changes there observed have been interpreted by Riddle as associated with *a progressive diminution of metabolism within the ova.*

It is further notable that the extent of seasonal change in thyroid size is greater in the common pigeons than in the other two kinds of birds This fact may be associated with the circumstance (see above) that the common pigeons were given least protection against cold in winter.

The data used in the construction of the polygons are given in table 3. Here also the body weights of the birds su¡ plying thyroid data (of the group aged 9 to 16.9 months) are recorded. The body weights of two other age-groups (7 to 8.9 months and 17 to 19.9 months) have been calculated and found, like the 9 to 16.9 months group, to be only slightly smaller during the "third" and "fourth" periods than in the "first" and "second" period. Though the body weight of the birds was somewhat reduced during the spring and summer periods (a normal change, long since known and reported—Riddle, 1916) the extent of this reduction is by no means commensurate with that observed in the thyroids.

CONSIDERATION OF EARLIER DATA, RELATED FACTS, AND DISCUSSION. A seasonal change in thyroid size has been demonstrated in three kinds of pigeons. In these cases it is thought that the means used in obtaining and analyzing the data exclude the possibility that the observed change is a spurious one ascribable to either of the following sources:

1. Unequal age distribution of the animals used at various seasons.
2. Unequal distribution of breed of animals used at various seaons.
3. The utilization of animals of unequal size at various seasons.
4. Differences in quality of diet and care at various seasons.
5. Differential distribution of thyroids from diseased animals.

Well-known facts make it clear that an apparent seasonal change, but really quite unrelated to season, might arise from any one of the above-mentioned sources.

The seasonal changes found in the three kinds of pigeons have the following time relations: An increase of thyroid size begins in the autumn and is either increased or maintained during the winter months. A

decrease in thyroid weight begins in the spring, or early in the year, and progresses to some point in the summer or early autumn. The months of larger thyroid size include October or November to March or April; months of smaller thyroid size include April or May to September or October. It is evident that in all these cases thyroid size promptly increases with cold weather and decreases with warm external temperature.

We must next consider how well or ill these time relations correspond with cases of seasonal change of thyroid size previously reported. On this point it is found that the data of Seidell and Fenger show: Largest thyroid size for sheep in May, June, April; smallest in February, December and March. For cattle, largest during April, May, March; smallest during August–January. For the hog (not considered as having shown a definite seasonal change of size), the largest thyroids were found during October, September, March and April; smallest during November–January. Seidell and Fenger have also presented curves for seasonal thyroid size in sheep as calculated from data earlier obtained by Martin and by Guyer. In these cases also the largest thyroids were obtained in May; smallest during July–January. Clearly our observations on birds fail by from two to four months to coincide with these data for mammals.

The amount of discrepancy just noted is far from negligible, and it is of much importance to know whether such differences really exist. In birds the response—increase or decrease in thyroid size—coincides closely with the changes in external temperature. The data for the mammals noted above indicate that this response is delayed by so long a period as two to four months. Since cattle, sheep and hogs supply practically all of the available information concerning thyroid size (at all seasons) in mammals we have taken very considerable pains to arrive at an opinion concerning the cause of this apparent difference of response in birds and mammals. This is the more desirable since the partial or incomplete data for the human and the frog seem to indicate a more immediate response, and thus to correspond better with our data for birds than with those already cited for mammals obtained at an abattoir. Though the conclusions at which we have arrived may be wrong it seems necessary to examine this matter quite fully.

We would first observe that a careful examination of the various publications by Martin, Guyer, Seidell and Fenger indicates the seasonal size variations observed by all these workers were notably influenced by one or another, or by a combination, of the four (possibly five) first mentioned factors which we have listed above. In the case of the sheep the particular factors that produce the appearance of seasonal variation

(apparently overlooked or but little considered by Seidell and Fenger) in the results have been well pointed out by Guyer—from whom we make the following quotations:

> In tabulation No. 1 it will be seen that there is a greater variation in the weight of the glands, ranging from 18 grains to 39.98 grains (two lots of glands taken four days apart in the month of June varied as follows: 235 lobes average 13.02 grains; 420 lobes, 25.00 grains), and although the monthly average weight seems to increase steadily (from December to June), a careful inspection of the tabulation will show that this is merely a coincidence rather than a real factor, as in each month some supplies were composed of large lobes and others of small lobes. . . . . This variation is accounted for by the fact that various flocks were killed, sometimes lambs, and at other times cross-breed, half-breed, Cheviots, etc. . . . .
>
> This marked variation in size was a distinct feature and one that called for further investigation. One consignment of 100 glands was examined, and each lobe weighed separately (table 3) and the weights were found to range from 11½ grains to 62 grains. . . . . To see how and why such great variations occurred as shown on the tabulations, two visits were made to the slaughter houses and I had the glands removed while present, and took full particulars of the class killed (table 4).
>
> Sixteen cross-breed lambs about 4–6 mo. old averaged 15.7 grains.
>
> Twelve sheep, 1 yr. old (Cheviots and cross-breeds), averaged 40.8 gr.
>
> Four 2-yr. old cross-breeds averaged 28.7 grains.
>
> It is this variation in the *age of the sheep* and *class of breed used* (italics are ours) which accounts for the great increase of lobe weight in March, April and May as then they were killing last season's lambs, or sheep about a year old.

We have already noted that in Scottish, English and American sheep the highest thyroid weights were obtained in May. Since most lambs are born in March or April and usually begin to appear on the market when five or six months old it is evident that glands taken from slaughter houses would begin to show unusual numbers of the small glands during September and October. These lambs would doubtless continue to appear in the market, but by the following March or April, these animals would be one year old. Lambs of one year, however, may have thyroids —according to Guyer—averaging 40.8 grains while those of the four to six-months lambs average only 15.7 grains. It will thus be seen that it is precisely the fall and winter months that average values obtained from random samples of sheep thyroids would be most affected by the inclusion of unusual numbers of younger animals. The correction of this error would evidently tend to advance the period of largest size by two to four months earlier in the year. This would bring the data for the sheep into fairly good agreement with those found in pigeons. It is also necessary to note that this large error would by no chance be avoided or overcome by the weighing of large numbers of sheep glands. Indeed it was Guyer, the investigator who weighed the largest numbers of glands (4000–7000 monthly), who found it possible and necessary to exclude season as the basis of the observed variations, and to point to *age* and *breed* as their true source.

Seidell and Fenger state (p. 78) as follows concerning Guyer's results:

> As will be seen, there is a very marked variation from day to day in the average weight per lobe, due in part no doubt to failure to reject abnormal glands or those from immature animals. In spite of this daily variation there is an unmistakable seasonal change in the average weight per lobe, and the maximum point corresponds approximately with that found for the same year by Martin, but is about one month in advance of the maximum point found by us for American sheep (the only published data of Seidell and Fenger on thyroid size—data for a year later—show *May* as the month of largest average thyroid size in sheep).

As opposed to the above, we are forced to believe that Guyer's results do not show a true seasonal variation, and that the interpretation which he gave of his own results is the true one. It seems wholly probable moreover that Guyer's interpretation applies also to the results obtained by Martin and by Seidell and Fenger on this same animal. The thick coat of wool of the sheep, and the frequency of its sudden removal (exposure to cold) by shearing in the spring, may be other complicating factors in sheep. Still further, the conditions affecting the marketing of cattle are rather similar to those already noted for sheep, and the data showing highest thyroid size in April—instead of two or three months earlier—may be similarly explained. Whether the appearance of smallest thyroids in hogs killed in November–January is due to this same circumstance is a matter upon which we have neither data nor an opinion. But differences in breed, in locality of origin, or in one or another aspect of the dietary and age of the animals, may here constitute the more important factor or factors. In any event the data for the hog—with October and March as the months of largest average thyroid size and showing June and July at very low points in the curve—are in rather better agreement with our own data than are those obtained for sheep. The total of the indicated seasonal variation in the hog is, however, quite small. Our data are most nearly in agreement with those obtained by Seidell and Fenger on cattle. If the high and low points in the seasonal curve for this animal came only two months earlier in the year, the agreement here would be satisfactory.

It must be stated that Seidell and Fenger recognized and considered the fact that variations exist from the above-mentioned, and from still other sources. They hoped, however, that their composite samples might so eliminate these factors as to make it possible to study the *seasonal variation in the iodine content* of the thyroids of these three kinds of animals. This last-named point was their special object of study. On this particular point we have made no studies whatever, and we wish to point out that, although we are convinced that their data do not give an accurate picture of the relation of *season* to *thyroid size*, we do not suppose that this circumstance affects to a similar degree the accuracy of their data for seasonal variation in iodine content. An animal aged

six months and another of two years may well have a similar percentage of iodine in any or all months of the year, though they would have glands very unequal in size. It may very well be, however, that other factors than age did considerably influence some at least of the iodine values found, and several of these factors were discussed at length by Seidell and Fenger. That race or breed may play a greater part than was then, or is now, recognized will be shown in a later paper by one of us. In still another place it will be shown that during the season of most active reproduction in pigeons the thyroids of both sexes are smaller, and the gonads are larger, than during the season of less active or of suspended reproduction.

With the following statements (p. 78) of Seidell and Fenger, statements evidently based chiefly on their iodine determinations, our own data for thyroid size and our conception of what is involved, are in good agreement:

> Among the several factors which may be considered of importance in accounting for the seasonal change in composition and activity of the thyroid gland may be mentioned temperature, diet, and periods of activity of the reproductive function. Of these the temperature effect in connection with its influence upon changes in metabolism appears to be of principal importance. During the colder months of the year the increased physical activity of animals together with the necessity for maintenance of the normal body temperature in a colder surrounding medium requires an increased metabolism which in turn would no doubt call for an increased output of the active thyroid material. Conversely, in the summer months, the demands upon the thyroid being less than the normal rate of production of active principle, would lead to a storing up of the iodine compound. The observed regular decrease in percentage of iodine beginning with the cooler weather in September and continuing through the winter to the warmer weather of early spring and the regular increase thereafter, appears to accord exactly with this reasoning.

In so far as thyroid size is concerned we find that our own data support the above statements rather better than do the similar data of Seidell and Fenger. For example, in their data it is difficult to observe any tendency of thyroid size during September, October and November to exceed the lowest summer value; such an increase, however, is found at this time in our data for all of the three kinds of pigeons. The extensive iodine determinations carried out by those observers—the values obtained from the thyroids of cattle particularly—do show a pronounced reduction of the iodine content during these autumn months.

In concluding this discussion of the true nature of the seasonal size variations of the thyroid in these mammals it may be admitted that a certain amount of difference is to be expected among various species of higher animals. In our own data, for example, a slight delay of the size increase apparently occurs in one kind of pigeon in contrast to the other two. Our examination of the mammalian cases, however, has indicated that both the apparent delay of hyperplasis in the autumn, and the ap-

parent continuance of largest thyroid size into the summer, in cattle, sheep and hogs, is apparent only and not real. Corrections probably necessary in those data tend to bring them into better agreement with the data obtained on birds. At some points, as last noted above, the iodine determinations made on the same material by Seidell and Fenger supply evidence that the gland weights as actually obtained do not properly represent the true time and extent of the change. The more extensive groups of data now available for both birds and mammals are thus in better agreement. They also now appear to agree with the more fragmentary data already cited in our introductory statement—namely, that human thyroids (Swiss) are larger in winter; that those from races of grass frogs from higher altitudes and colder regions are larger; that frogs reared in the cold have larger thryoids; and that the thyroids of mammals subjected to relatively *short periods* of heat show size reduction, while those exposed to cold show histological changes characteristic of hyperplasis.

There is other evidence concerning seasonal variation of iodine content, or of functional activity, than that already cited. Little will be said of this evidence here, since it is rather indirectly related to questions of thyroid size; but it should be mentioned that some of these data do not well accord with our own results nor with our interpretation of them as already given. So far as we are aware, Koch (1907) was the first to report seasonal differences in the iodine content of the thyroid. Larger amounts were found "during the winter months than during June and July." Koch, however, did not definitely conclude that such seasonal variations really exist, and age differences of the animals were cited as a probable factor of much importance. Again, Hunt (1910), using the acetonitril test, seems to have been the first to obtain data throughout the year on seasonal variations in the functional activity of the thyroid. He found such a variation in mice and guinea pigs despite the fact that they were kept at "fairly uniform" temperatures. The guinea pigs were most resistant to acetonitril poisoning in spring and summer—the period found by us to be the period of decreasing thyroid size. The mice were least resistant during this same period. This result with mice conforms to the generally unique behavior of mice to acetonitril (Hunt, 1923).

Though the possible influence of diet can not be readily excluded as a basis for the apparent effect of season in the case of most of the animals earlier investigated and discussed above, it is probably sufficiently excluded in the case of the three kinds of pigeons reported here. The relations of one or another aspect of diet to the activity and to hyperplasis of the thyroid are among the best known facts of thyroid physiology. We have referred to this well-known relationship only as it is conceivably responsible for variations which have been attributed to

season. It therefore seems beyond the limits of this paper to refer to the numerous contributions made to this topic by Watson, Hunt, Marine, Bensley, Jackson, McCarrison, Cramer and others.

The question of "species variability" touched upon in a preceding paragraph, requires a further statement. The thick coat of wool of the sheep, and the sudden exposure of sheared animals to the effects of cold, have been cited as conditions probably capable of disturbing the normal seasonal changes in thyroid size in this animal. It further seems probable that in some much mongrelized domestic animals, in poultry particularly, other disturbing conditions may have been established in the form of racial characteristics. Most breeds of the common fowl, for example, may freely and normally lay eggs in mid-winter—though the less changed Jungle fowl and practically all wild species of birds do not. It is also probable that the winter-laying domestic breeds now have, as a heritable racial characteristic, the capacity to produce a more abundant and effective feathering than have the pure original species from which they have been derived. Again, the racial characteristic underlying this possibility of mid-winter reproduction—not present at least in similar degree in pigeons given no protection from cold—might rest upon a very strong immediate response of the thyroid to the cold weather of autumn; a response sufficient (by increasing the body covering, or otherwise) to permit an early (winter) partial decline of thyroid size coincident with egg-laying here, as we find to hold in pigeons. Our point here is to indicate certain types of difference which probably exist in animals, and to suggest that these divergences may exist without violence to the real point of the general rule. For example, the hibernating mammals—with atrophied thyroids in winter—confirm the rule, rather than oppose it, by their hibernation. Possibly, however, real violations of the rule will be found. Most angles of this question would seem to turn principally upon relations of the thyroid to the mechanism of heat regulation.

The present data contribute a further item of evidence that the thyroid functions as a part of the heat-regulating mechanism. To the earlier evidence of the same kind adduced by Seidell and Fenger is added the evidence from the coördinate group of warm-blooded vertebrates (birds). Also, the present results make it probable that the thyroids of mammals respond by an increase in size more quickly than was indicated by the data of the earlier investigators who have made weighings covering the entire year. A similar response to heat and cold by the thyroid of the frog suggests that the influence of the thyroid on the rate of oxidations is exercised throughout the vertebrate series of animals. Finally, whether mere coincidence or otherwise, the one kind of pigeon which was subjected to severest winter temperatures is the species in which the greatest amount of seasonal variation was found.

A word may be added concerning the relation of sex and body weight to thyroid size. No detailed analysis of this matter has been attempted, but it is entirely clear that in any single season-and-age unit of our material the thyroid size is very much more variable than is the body weight. The greatest variability is undoubtedly most often found in individuals with thyroids larger than the average or mean; though one case of almost complete absence of thyroids, and two cases of a single gland instead of a pair, are included in our data. An essential equivalence of thyroid size in the two sexes (except for older females) is indicated, as already noted, but the data are not sufficient to permit a final statement. On both of these points our data are in accord with Hammett's (1924) findings on albino rats aged 150 days. It was there found that thyroid weights below the mean were positively correlated with the body weight; those above the mean showed a negative correlation. No sex difference was found in the rat.

One factor conceivably related to the observed seasonal changes in thyroid size remains to be considered. The period of largest thyroid size falls in the winter—when the hours of sunshine and daylight are fewest; smallest thyroids are found in summer—when the daily period of sunshine is longest. May it be therefore that changes in thyroid size are responses to the relative amounts of light and darkness? It would be quite impossible to answer this question from our own data. Fortunately, however, the studies cited above show that the thyroids of mammals and frogs enlarge when subjected to low temperatures and decrease in high temperatures. Those results can not run parallel to the length of day, but they do fully coincide with the temperature changes of the seasons. The available evidence therefore supports the conclusion that the seasonal size changes observed by us are effected by seasonal changes in temperature. This conclusion, of course, is in no way contradictory to the well-known fact that other factors also are capable of increasing and decreasing thyroid size. Brown, Pearce and Van Allen (1924) have found that some of the organs of the body (the thyroid is not included in the report) not only undergo seasonal size changes, but in some cases these changes are more closely correlated with the amount of monthly sunshine than with season.

The preceding examination of available facts concerning seasonal size changes in the thyroid gland suggests and supports the following generalization: Normally and typically the thyroids of higher vertebrates respond promptly, by enlargement, to the onset of lower temperatures in the autumn and winter, and by a size decrease in the spring and summer; these size responses may be initiated by temperature changes alone; and cases similar to hibernation in mammals are doubtless to be regarded as intelligible exceptions to this normal rule.

It remains to point out briefly the relation of the facts developed here to seasonal differences in the sex ratio, in grade or degree of sexuality, and in some notable reproductive processes, as these have been previously observed in pigeons. Whatever may be the value of the present results to other problems the numerous data on the above topics, as obtained by Riddle during several preceding years, make it entirely clear that these results go far toward supplying the facts necessary to an understanding of those seasonal changes in sex and reproduction.

In the earlier studies of Whitman (1919) and Riddle (1914, 1916) it was found that from pigeons made to produce many eggs during the reproductive season there arise more males from the eggs of the early season (spring) and more females later in the season or year. Also, that the winter is usually a period of interrupted reproduction—ceasing altogether in these species unless given some protection against cold. Further (Riddle and Honeywell, 1924), that the onset of cooler weather in the autumn is the beginning of an interruption (marked by longer intervals between ovulations, "single" instead of paired eggs, etc.) which usually precludes the successful continuance of rapid egg-production ("reproductive overwork"; "crowded reproduction") beyond this period. Relatively masculine females (as measured by copulatory behavior with other females, and by other tests) were obtained from certain crosses and these were found by Riddle (1916) to arise more frequently in early than in late season; conversely, the most feminine females (as shown by copulatory behavior and the frequency of persistent right ovaries) were found to arise in greater frequency from the later part (summer) of the season of reproduction. The male-producing eggs of the early season were found to be smaller—to have *stored* less material and energy—than the female-producing eggs developed later in the summer. From the evidence afforded by the comparative storage powers of these two kinds of eggs, and from other confirmatory kinds of evidence, Riddle has interpreted the male-producing ova of spring as ova possessing a higher metabolic rate—as cells that oxidize more of the ingested material and store less of it; while female-producing ova of the later season are cells of a lower metabolic level—oxidizing less of ingested material and increasing their bulk by storing more of it.

When the present data for seasonal change in thyroid size in these animals (as seen in the polygons of figure 1 and in other tabulations) are united with those earlier results the following associations appear: The season of reproduction in these animals coincides with the period of *decrease in thyroid size*—February or March to August. The period at which rapid egg-production is interrupted—usually in September, though less often beginning in August—is a period of beginning *increase in thyroid size.*

The seasonal relations of the changing sex ratios to thyroid size are of still greater interest. The part of the season of most abundant male-production (spring) is the part of the reproductive season characterized by *largest thyroid size* in the parents. The female-producing part of the season is that part of the entire year characterized by *smallest thyroid size* in the reproducing parents. In view of the fact that largest thyroid size is here clearly associated with the highly functioning thyroid of winter, and that increased oxidations (metabolism) result from this functioning, there can be scarcely a doubt that the larger thyroids of early spring are associated with a relatively high metabolism in the pigeon's body at this period; and, further, as little doubt that the smallest thyroids of the summer period are likewise associated with a reduction of the metabolism at this period of maximum production of females from the ova then formed.

The agreement of the present results with the facts earlier obtained by the above-named observers, and with the interpretation earlier given them by one of us, is therefore gratifyingly good. It would, however, probably be wrong to assume that under all circumstances the metabolic level of the body (soma) is parallel to that of the germinal tissue (ova). The application of these results to the numerous individual records for sex ratios as previously obtained will be made in detail elsewhere by the senior author. The further and complete agreement of the thyroid-sex data in birds, with the similar thyroid-sex data obtained by Adler in frogs, is a most satisfactory outcome of the large amount of work that has been done on experimental modifications of sex in these two groups of animals. At the same time, the known relations of the thyroid to metabolism now provide direct evidence for the interpretation—first made by Riddle (1914)—of differential sex development on the basis of differential metabolic rates.

SUMMARY

Three kinds or species of pigeons were kept on the same diet throughout the year and killed during all months of a three-year period. The weights of the thyroids from these three species indicate a nearly simultaneous enlargement in autumn and winter months and a progressive decrease in size during the months of spring and summer.

These size changes promptly follow the onset of colder autumn, and warmer vernal temperatures. Promptness of change, rather than evident delay, is indicated by practically all of the data obtained.

This seasonal enlargement of the thyroid is probably associated with seasonal increase of thyroid function and increased heat production.

The changes as measured are probably essentially free from the complicating effects of variations in the diet, care, locality, race, age, length

or degree of confinement, health, or body size of the animals used in the study.

Earlier studies on seasonal changes in thyroid size, and on size changes induced by subjection of animals to short periods of heat and cold, have been considered at length. Some probable inaccuracies of the data previously obtained on seasonal changes in thyroid size of mammals have been pointed out. Data of these two kinds have been shown to be related, and evidently in better accord than was hitherto apparent.

This considerable body of earlier and present data suggests and supports the following generalization: Normally and typically the thyroids of higher vertebrates respond promptly to autumn temperatures by enlargement, and to spring and summer temperature by a decrease in size; and these responses are capable of being induced by the temperature alone.

The seasonal changes in thyroid size observed in pigeons have a direct and important application to the seasonal changes in sex ratios and in reproductive processes as these have been previously observed in this laboratory. The season of active reproduction in the pigeon covers the period of decline of thyroid size—from its peak to its lowest point. The period of excess of male offspring coincides with the period of larger thyroid size; the period of excess of females is coincident with the period of smallest thyroid size.

These results in the pigeon are also in full agreement with the thyroid-sex data obtained by Adler on frogs. They supply new evidence that in both bird and frog the successful experimental modifications of sex differentiation have been effected through changes in the rate of metabolism in the ova and early embryos.

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AUGMENTATION OF THE VASCULAR RESPONSES OF THE CAT TO SUCCESSIVE VASOMOTOR STIMULI OF EQUAL VALUES[1]

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Received for publication February 24, 1925

The blood pressure method for the physiological assay of preparations of adrenalin has long been used because of the apparent similarity between the vascular responses to successive equal doses. Numerous modifying factors have, however, been pointed out which must be carefully controlled in using the method. Among these factors are changes in the velocity of the injections, the amount of ventilation, the depth of anesthesia, the blood volume, the H-ion concentration of the blood, the injection of tissue extracts and so on. With these factors controlled, a progressive augmentation of the pressor responses following equal doses of adrenalin was described by Levy in 1916 (1) which he attributed to the effect of a secretion from the thyroid. This augmentation appeared between 40 and 60 minutes after stimulation of the thyroid or after the injection of thyroxin and was sometimes as much as 300 per cent. Lieb and Hyman in 1922 (2) confirmed Levy's findings but they also found that the augmentation appeared in cats in which the thyroids had been extirpated several days before the experiment. They concluded that the augmentation was due to a sensitization of some part of the autonomic nervous system, probably of the myoneural junctions. Hunt in 1918 (3) noted the same phenomenon in cases where the thyroid had been removed some hours previously and suggested that it might be due to a gradual loss of irritability of the vasodilator mechanism. Dryerre (4) has recently reported that he failed to get augmentation after injecting thyroid extract and thyroxin.

The present paper attempts to set forth the conditions under which the phenomenon of augmentation occurs, to show how it can be produced or prevented, and to offer an explanation.

Unless otherwise specified the cats were anesthetized with ether for a brief period during which a tracheal cannula was inserted and both vagi were cut. The brain and medulla were immediately pithed through the

[1] This investigation was begun in 1916 in the Physiological Laboratory of the Harvard Medical School. In 1917 it was interrupted by the World War and was continued in 1922 at Boston University.

orbit. Then the cord was pithed by stages to various levels, even to complete destruction in some cases. The body temperature was kept constant by means of an electric heating pad. Adrenalin chloride solution (Parke, Davis & Company), usually diluted to one part in 100,000 parts of tap water, was injected into a femoral vein. The dose was usually 0.5 cc. but in some cases smaller amounts were used. The rate of injection was controlled by injecting 0.1 cc. a second synchronously with the beat of a metronome. When the splanchnic nerves were stimulated shielded platinum electrodes were used. The blood pressure was recorded by means of a mercury manometer connected with a carotid artery. The anticoagulant was either 10 per cent sodium citrate or sodium carbonate (sp. gr. 1.08) but the sodium citrate was found to be more satisfactory. Any effects due to passage of the anticoagulant into the blood stream during a fall of blood pressure were controlled but it was found that as a rule special precautions were unnecessary. Blood for CO_2 capacity determinations was drawn under paraffin oil from a femoral artery either before or after pithing and at intervals of 20 minutes to an hour throughout the experiment. The CO_2 capacity of the plasma was measured by the Van Slyke method and of the whole blood by the Henderson-Morriss method. Whenever it was desired to obviate the effects of an anesthetic the cat was stunned instantaneously, without previous anesthetization, by a blow on the head, and the brain was immediately pithed through the orbit. This was done so as to produce immediate unconsciousness without stopping natural respiration. Then a tracheal cannula was quickly inserted, artificial respiration administered, and the pithing continued.

When successive equal doses of adrenalin, 10 or 15 minutes apart, were injected into a cat which had been etherized, pithed and prepared for registering blood pressure, the rises of blood pressure following each injection were not equal in magnitude. In the majority of cases there occurred an augmentation of the vascular responses which began to appear in about 2 hours after pithing, reached a maximum in about 3 hours and began to disappear in from 3 to 4 hours after pithing. The amount of augmentation varied between 30 and 600 per cent of the initial responses, being about 100 per cent in the majority of cases. Moreover, if the splanchnic vessels were tied off and the secretory nerve to one adrenal was stimulated electrically with equal strengths of stimulus a similar augmentation of the vascular responses to the secreted adrenalin occurred (fig. 1). When one adrenal was tied off and the corresponding splanchnic nerve was stimulated a similar augmentation of the direct vasomotor responses occurred (fig. 2). These responses, due only to electrical stimulation of vasoconstrictor nerves, were easily distinguished from adrenalin responses. When one test dose was given at the beginning of an experiment and no more was given until three hours after pithing, a single injection at that time pro-

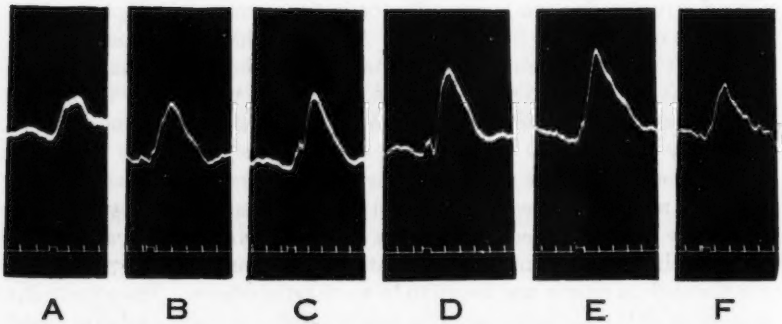

Fig. 1. Experiment 16. Cat etherized and pithed through brain to low cervical cord. Coeliac, superior mesenteric and inferior mesenteric arteries ligatured. Electrical stimulations of left splanchnic nerve for 15 seconds each at a rate of 10 induction shocks per second. Pressor responses due to adrenalin secreted in response to stimulations. Base line records time in half minutes and is at zero blood pressure level. Blood pressure in *A*, before stimulation, is 54 mm. Hg. Time after pithing: *A*, 54 minutes; *B*, 119 minutes; *C*, 160 minutes; *D*, 174 minutes; *E*, 193 minutes; *F*, 220 minutes. Pressor response of *E* is 150 per cent greater than that of *A*.

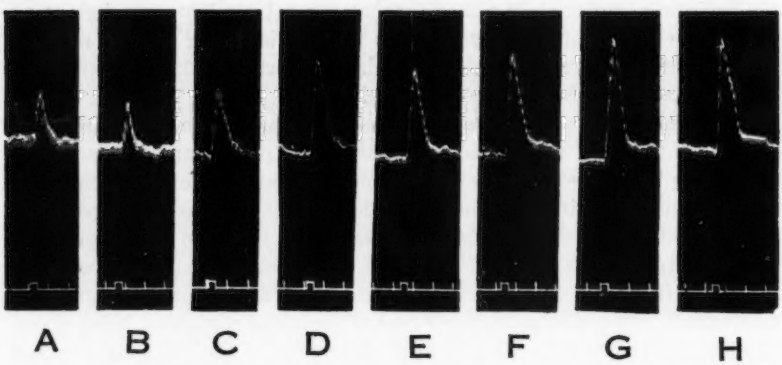

Fig. 2. Experiment 31. Cat etherized and pithed to high thoracic cord. Left adrenalectomy. Electrical stimulations of left splanchnic nerve for 10 seconds each at a rate of 10 induction shocks per second. Pressor responses due to electrical stimulations of vasomotor nerves. (Note rapid rise and fall of blood pressure as compared with that due to adrenalin in fig. 1.) Signals as in figure 1. Blood pressure in *A*, before stimulation, is 64 mm. Hg. Time after pithing: *A*, 100 minutes, *B*, 135 minutes; *C*, 197 minutes; *D*, 210 minutes; *E*, 227 minutes; *F*, 237 minutes; *G*, 248 minutes; *H*, 260 minutes. Pressor response of *G* is 178 per cent greater than that of *A*.

duced a typical augmented rise of blood pressure. Lieb and Hyman (2) reported obtaining an augmentation after double adrenalectomy. Such results showed that the augmentation is not specific for injected adrenalin, that it is not due to secreted adrenalin nor to an accumulation of adrenalin, but that it is a peculiarity of pure vasomotor responses.

The amount of pithing did not affect the usual course of events. The augmentation appeared in animals in which the brain alone had been destroyed and in others which had been pithed to various levels of the

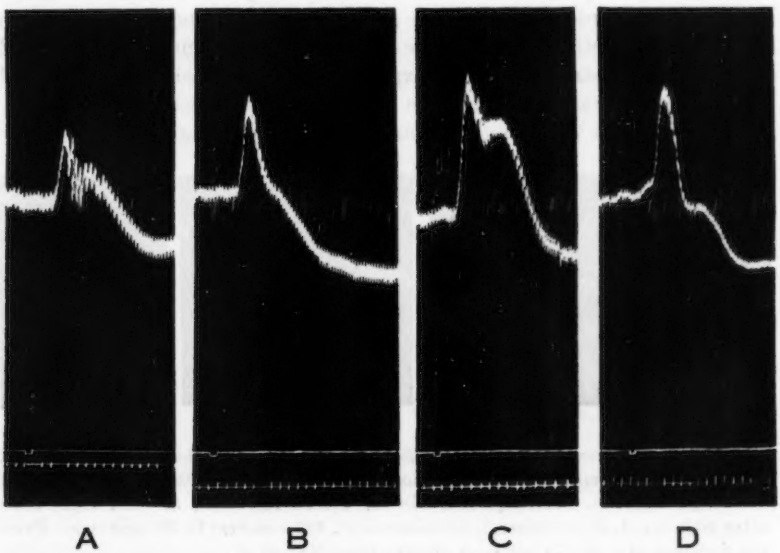

Fig. 3. Experiment 70. Urethane (2 grams per kgm. of body weight) by stomach tube at 11:30. Adrenalin chloride injections (dose: 0.3 cc. of 1:10,000 at the rate of 0.1 cc. per second). In this, and in all subsequent tracings, the injection signal line is at zero blood pressure level and the time record shows five second intervals. Blood pressure in *A*, before injection, is 116 mm. Hg. Time of injection: *A*, 3:39; *B*, 5:42; *C*, 8:16; *D*, 9:25. Pressor response of *C* is 114 per cent greater than that of *A*.

cord, even to complete destruction of the central nervous system. In fact it was not necessary that the animal be pithed at all, because a typical augmentation was obtained in an animal under urethane anesthesia (fig. 3). Most of the animals used in these experiments were pithed, however, to get rid of influences from the vasomotor centers and to allow an accurate control of the respiration.

In the pithed cats artificial respiration, constant in rate and volume, was employed. In some cases the ventilation was reduced so that there was

just enough to keep the animal alive, or increased so that the cat was obviously over-ventilated throughout the experiment; and under both conditions an augmentation appeared as usual. It appears, therefore, that the amount of ventilation as administered in these experiments was not in itself responsible for the augmentation. It was customary to adjust the ventilation at the beginning of the experiment so that the blood appeared to be sufficiently oxygenated.

In another investigation we noted that the response to adrenalin could be varied by varying the amount of ether given just before or during the injection. In order to see if the augmentation was not merely due to recovery from ether, experiments were done by stunning the cat and pithing immediately. In such experiments an augmentation appeared earlier than in cases in which the cat was anesthetized before pithing, that is, in from 30 minutes to an hour after pithing. Otherwise the

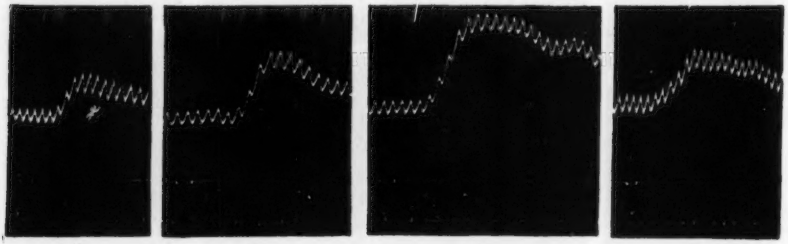

Fig. 4. Experiment 77. Cat stunned and pithed to low thoracic cord without anesthesia. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000 at the rate of 0.1 cc. per second). Blood pressure in *A*, before injection, 52 mm. Hg. Time after pithing: *A*, 55 minutes; *B*, 140 minutes; *C*, 193 minutes; *D*, 293 minutes. Pressor response of *C* is 173 per cent greater than that of *A*.

phenomenon was the same (fig. 4). Evidently the augmentation is not a manifestation of recovery from ether but its onset may be delayed an hour or two by the preliminary anesthetization.

The occurrence of the augmentation was not dependent upon the existence of any particular level of blood pressure. It appeared in cases in which there was a constant level of blood pressure as low as 30 mm. of mercury on the one hand and as high as 130 mm. on the other. Since most of the animals used in these experiments were pithed the augmentation was obtained quite regularly with blood-pressure levels between 30 and 80 mm. of mercury, with a pressure from 50 to 60 mm. as an average. An augmentation sometimes occurred when the blood pressure was fairly high at the beginning and fell slightly during the course of the experiment, but it did not occur when the blood pressure was relatively low and then fell progressively.

Double thyroidectomy was done in a majority of the experiments so that Lieb and Hyman's (2) conclusion that the augmentation does not depend upon a sensitization produced by a secretion from the thyroid was amply confirmed. The criticism that the operation of thyroidectomy might cause sufficient outpouring of secretion to produce the phenomenon

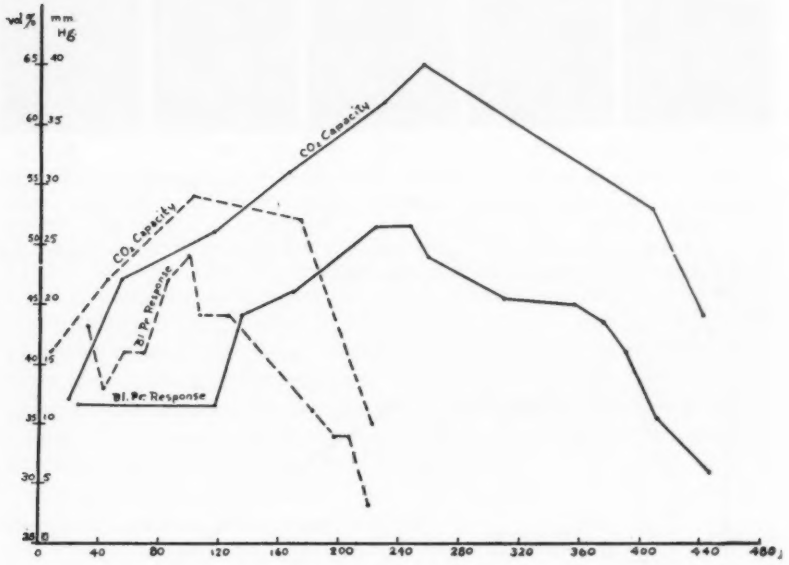

Fig. 5. Experiments 81 (broken lines) and 83 (solid lines). Correlation between the vascular responses and the CO_2 capacity of the blood. Responses augmented. In this and in subsequent charts ordinates represent CO_2 capacity in volumes per cent and blood pressure in millimeters Hg., abscissae time after pithing in minutes. Each point on the blood pressure curve represents the height of the vascular response to an injection of 0.5 cc. of adrenalin chloride, 1:100,000. Each point on the CO_2-capacity curve represents the CO_2 capacity of a sample of whole blood. Cat etherized and pithed to mid-cervical cord in experiment 81 and to mid-thoracic cord in experiment 83. In experiment 81 the blood pressure remained at about 110 mm. Hg. until 126 minutes after pithing and then fell gradually to 20 mm. Hg. at the end. In experiment 83 the blood pressure remained between 75 and 80 mm. Hg. until 356 minutes after pithing and then fell gradually to 36 mm. Hg. at the end.

was controlled by performing thyroidectomy under ether five or six hours before the experiment was begun and allowing the cat to recover from the anesthetic. A typical augmentation was obtained in the usual time after pithing although sufficient time had lapsed between thyroidectomy and the beginning of the experiment to have allowed the augmentation to

appear and disappear if it had been produced by a secretion from the thyroids.

It was found, however, that a correlation exists between the vascular

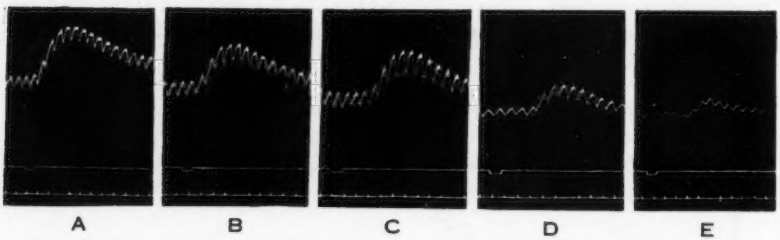

Fig. 6. Experiment 79. Cat stunned and pithed to high cervical cord without anesthesia. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000). Blood pressure in *A*, before injection, 63 mm. Hg., in *E*, 40 mm. Hg. Time after pithing: *A*, 43 minutes; *B*, 79 minutes; *C*, 133 minutes; *D*, 178 minutes; *E*, 218 minutes. No augmentation of the pressor responses occurred.

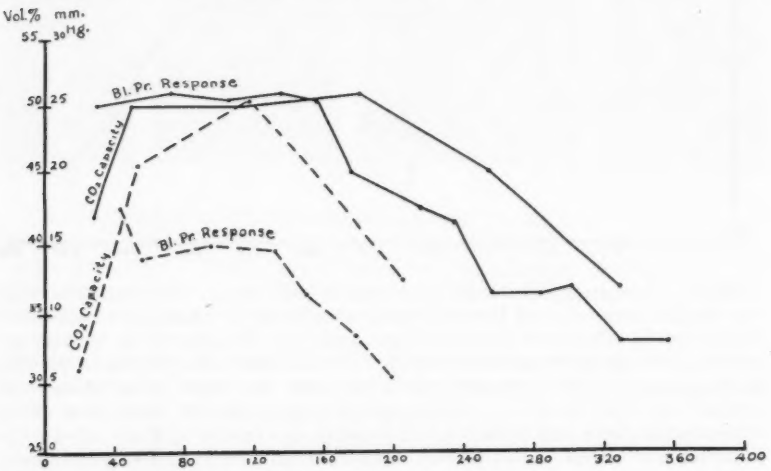

Fig. 7. Experiments 79 (broken lines) and 82 (solid lines). Correlation between the vascular responses and the CO_2 capacity of the blood. Responses not augmented. Cat stunned and pithed to high cervical cord without anesthesia in experiment 79; etherized and pithed to high cervical cord in experiment 82. In experiment 79 the blood pressure remained at about 62 mm. Hg. until 133 minutes after pithing and then fell gradually to 40 mm. Hg. at the end. In experiment 82 the blood pressure fell gradually from about 78 mm. Hg. at the beginning to 23 mm. Hg. at the end. (See figure 6 for tracings of experiment 79.)

responses and the CO_2 capacity of the blood. At the beginning of the experiment, just after pithing or just after discontinuing the ether, the

CO_2 capacity was usually between 35 and 45 volumes per cent. This, we believe, can be considered low for a cat. During the first hour or two, while the response to adrenalin remained about the same, the CO_2 capacity increased slightly to between 45 and 50 volumes per cent. During the next hour, as the vascular responses began to increase, the CO_2 capacity increased also to between 50 and 55 volumes per cent. As the augmentation reached its maximum the CO_2 capacity was usually between 55 and 60 volumes per cent. Then, as the responses began to decrease, the CO_2 capacity, instead of decreasing also, either remained at the same high level, or increased still further, sometimes to 65 volumes per cent or more. By the time the vascular responses had reached their previous low level, or just before that time, the CO_2 capacity had begun to decrease and had reached an average of 50 volumes per cent. Later the CO_2 capacity decreased still further to 40 or 45 volumes per cent, or even lower, and this was usually followed by the death of the animal (fig. 5).

Although an augmentation occurred in the majority of cases, there were some in which the responses decreased progressively from the beginning of the experiment until death, which occurred relatively early, or else the responses remained of about the same magnitude for two or three hours after which they decreased progressively to the end (fig. 6). It was noted that in such experiments the blood pressure was low and progressively falling. In all cases where an augmentation failed to appear the CO_2 capacity did not rise above 51 volumes per cent. It usually started at about the same level as in cases where the augmentation did appear, and increased slightly during the first hour to between 45 and 50 volumes per cent; but it remained at this level throughout the experiment and fell below 40 volumes per cent at the end. At no time did the CO_2 capacity rise to the level at which augmentation appears in other experiments (fig. 7).

In view of the correlation between the CO_2 capacity of the blood and the relative ability of the vasomotor system to respond to stimulation, an attempt was made to alter the vascular responses by changing the CO_2 capacity by the injection of buffer salts and acids. This we were able to do in a few cases, thus confirming the work of Collip (5). It was found that upon injecting an appropriate amount of 25th normal HCl (about 5 cc. per kgm.) at the beginning of an experiment the CO_2 capacity remained between 40 and 50 volumes per cent and the response remained constant in height for more than 3 hours. Following this, an injection of 5 per cent sodium bicarbonate (about 5 cc. per kgm.) produced a rapid increase of the CO_2 capacity accompanied by a 100 per cent augmentation of the pressor responses (figs. 8 and 9). About 10 cc. per kgm. of normal sodium bicarbonate injected at the beginning of an experiment caused a rapid increase of the CO_2 capacity from about 40 to 60 volumes per cent accompanied by

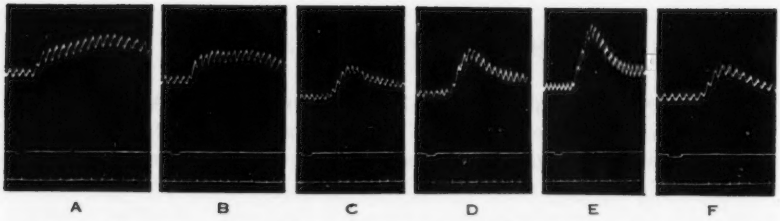

Fig. 8. Experiment 87. Effect of injections of HCl and $NaHCO_3$ on vascular responses to adrenalin. Cat etherized and pithed to mid-thoracic cord. Adrenalin chloride injections (dose: 0.5 of 1:100,000). Blood pressure in *A*, before injection, 78 mm. Hg. Time after pithing: *A*, 28 minutes; *B*, 84 minutes; *C*, 178 minutes; *D*, 184 minutes; *E*, 234 minutes; *F*, 272 minutes. Between *A* and *B*, 34 minutes after pithing, 15 cc. N/25 HCl were injected at the rate of 1 cc. per minute. After *C*, at 179 minutes after pithing, 15 cc. of 5 per cent $NaHCO_3$ were injected at the rate of 1 cc. per minute; after *D* at 224 minutes after pithing 30 cc. of $NaHCO_3$ were injected.

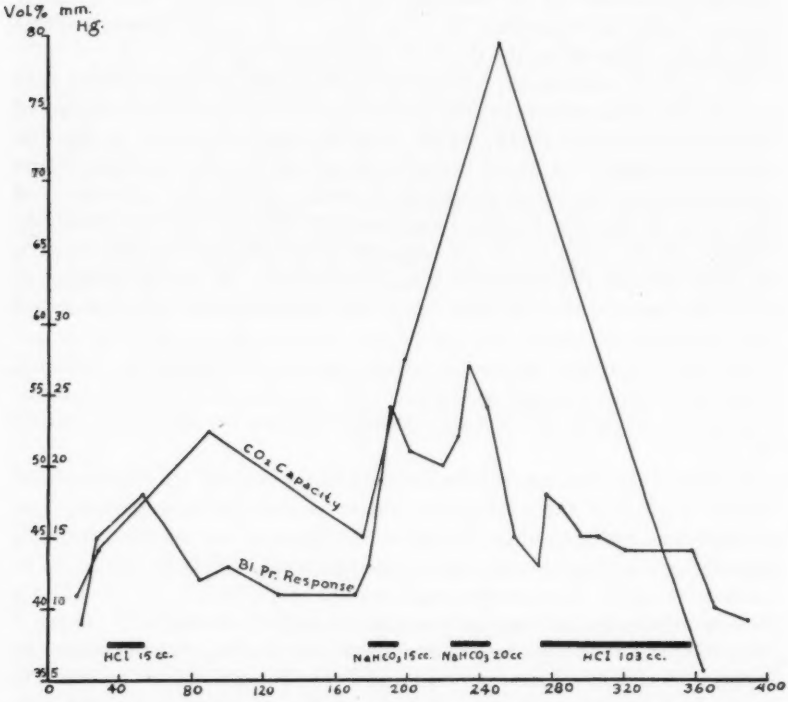

Fig. 9. Experiment 87. (See fig. 8 for tracings.) Correlation between the vascular responses and the altered CO_2 capacity of the blood. The time and amount of injections of N/25 HCl and 5 per cent $NaHCO_3$ are indicated on the base line (rate of injection 1 cc. per minute). Cat etherized and pithed to mid-thoracic cord. The blood pressure was about 78 mm. Hg. until 84 minutes after pithing. Then it fell to about 60 mm. Hg. and remained at this height throughout the experiment.

a 33 per cent augmentation of the vascular responses which appeared between 40 and 50 minutes after pithing, reached a maximum in from 10 to 20 minutes, and disappeared in about half an hour. The CO_2 capacity later decreased to a low point but the responses had decreased to a low level before the CO_2 capacity had begun to decrease (figs. 10 and 11; expts. 88 and 92). The injections of the buffer salt and the acid were given at the rate of 1 cc. per minute and at body temperature. Under these conditions there was no change in the original level of blood pressure. Control experiments showed that the same amount of Ringer's solution injected at the same rate produced little or no increase of the pressor response to adrenalin (fig. 11, expt. 99 and fig. 12). A transient increase

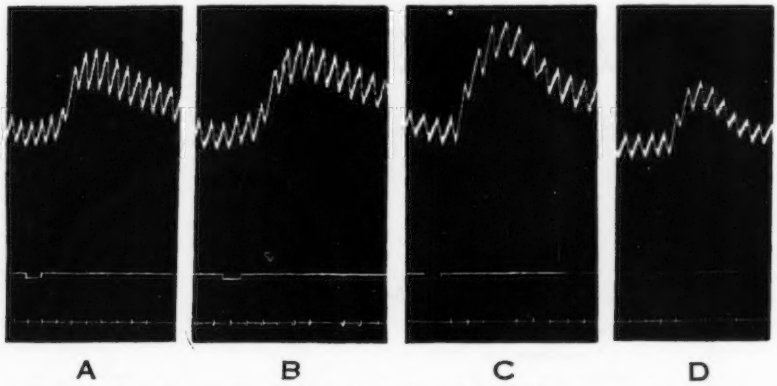

Fig. 10. Experiment 88. Effect of injection of $NaHCO_3$ on vascular responses to adrenalin. Cat etherized and pithed to low cervical cord. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000). Blood pressure in *A*, before injection, 78 mm. Hg. Time after pithing: *A*, 43 minutes; *B*, 49 minutes; *C*, 68 minutes; *D*, 78 minutes. After *A*, at 45 minutes after pithing, 30 cc. of normal $NaHCO_3$ were injected at the rate of 1 cc. per minute.

of the pressor response to adrenalin can be produced, however, by the injection of a relatively small amount of fluid at more rapid rates. Thus it appears that the augmentation does not occur when the CO_2 capacity is kept low by the injection of acid, and it may appear either earlier or later than it usually does when the CO_2 capacity is increased by the injection of sodium bicarbonate (fig. 11, expts. 88, 91 and 92).

The fact that the vascular responses began to decrease before the CO_2 capacity had reached its maximum indicates that there is an optimum CO_2 capacity for vasomotor activity. This appears to be in the neighborhood of 55 volumes per cent. This is significant in the light of the work of others. Hammett (6) and Evans and Underhill (7) showed that increase of alkalinity of the surrounding fluid favors the contraction of smooth

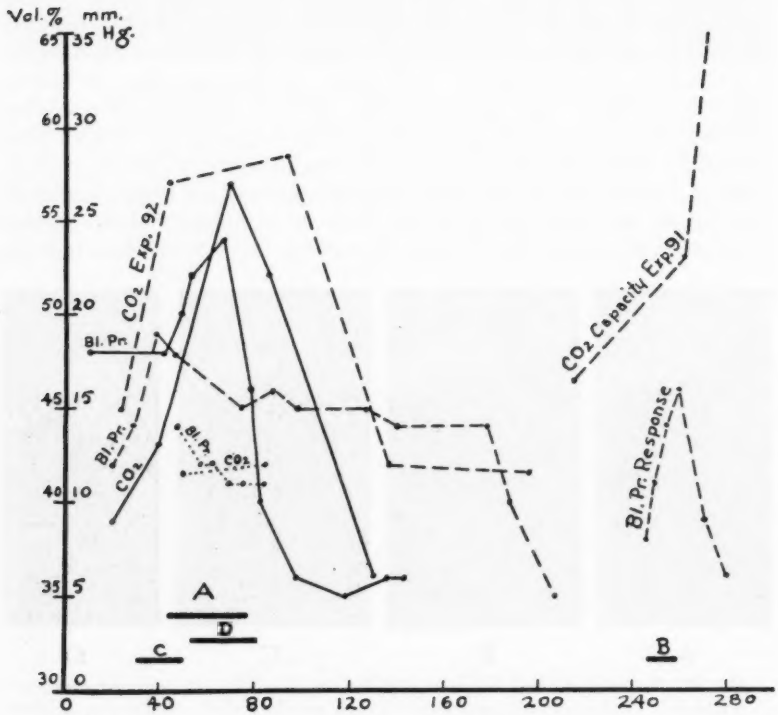

Fig. 11. Experiment 88 (solid lines. See fig. 10 for tracings). Correlation between the vascular responses and the altered CO_2 capacity of the blood. Cat etherized and pithed to low cervical cord. At *A* 30 cc. of normal $NaHCO_3$ were injected. The blood pressure fell gradually from about 90 mm. Hg. at the beginning to about 50 mm. Hg. at the end.

Experiment 91 (broken lines). Cat etherized and pithed to mid-thoracic cord. At *B* 10 cc. of normal $NaHCO_3$ were injected. The blood pressure was about 40 mm. Hg.

Experiment 92 (broken lines). Cat etherized and pithed to mid-thoracic cord. At *C* 15 cc. of normal $NaHCO_3$ were injected. The blood pressure remained between 60 and 75 mm. until 128 minutes after pithing and then fell gradually to 34 mm. Hg. at the end.

Experiment 99 (dotted lines. See fig. 12 for tracings). Absence of effect of fluid injection on vascular responses and CO_2 capacity. Cat etherized and pithed to high thoracic cord. At *D* 25 cc. of Ringer's solution were injected. The blood pressure was about 55 mm. Hg.

muscle whereas increase of acidity favors relaxation. Moreover Snyder and Andrus (8), and Snyder and Campbell (9) have shown that the effect of adrenalin on the heart of the terrapin and the perfused vessels of the frog depends upon the pH of the perfusing fluid. An increase of acidity caused a loss of tonus of the heart and a dilatation of blood vessels while a decrease of acidity caused an increase of tonus of the heart and a constriction of blood vessels. Adrenalin given under these conditions augmented these effects. Collip (5) also reported that the pressor effect of adrenalin in the dog is increased by decreasing the blood acidity by injecting Na_2CO_3, and is decreased by increasing the blood acidity by injecting acid sodium phosphate. Thus it might be expected that changes in the composition of the blood resulting from disturbance of the circulatory conditions would cause changes in the reaction of the fluids surrounding the smooth muscle

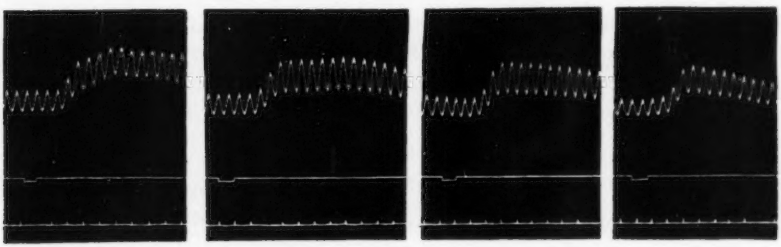

Fig. 12. Experiment 99. Absence of fluid effect on vascular responses to adrenalin. Cat etherized and pithed to high thoracic cord. Adrenalin chloride injections (dose: 0.5 cc. of 1:100,000). Blood pressure in *A*, before injection, 58 mm. Hg. Time after pithing: *A*, 48 minutes; *B*, 57 minutes; *C*, 69 minutes, *D*, 83 minutes. After *A*, at 54 minutes after pithing, 25 cc. of Ringer's solution were injected at the rate of 1 cc. per minute.

of the blood vessels, and consequently variations of the vascular responses. It is possible that the CO_2 capacity is an expression of the pH index of the tissue fluids in the smooth muscle and that 55 volumes per cent is correlated with the optimum pH for smooth muscle activity. It is also possible that there are changes in the ability of the tissues to utilize oxygen under the abnormal circulatory conditions and that the changes in CO_2 capacity are indicative of these changes.

The heart rate showed no appreciable alteration which could be correlated with the augmentation of the pressor responses or with the changes in CO_2 capacity. In fact it was found to be remarkably constant throughout any one experiment. The accelerations of the heart rate produced by the equal doses of adrenalin were also constant. This indicated that there was no general sensitization of the myoneural junctions of the thoracicolumbar division of the autonomic nervous system as Lieb and Hyman (2)

suggested. If such a sensitization had occurred one would have expected to find an augmentation of the acceleration of the heart rate inasmuch as adrenalin is supposed to act on the myoneural junctions of the accelerator nerves. Therefore it seems probable that the augmentation was due to an increased ability of the smooth muscle to respond to stimulation.

It is evident that under the conditions which exist in a study of vascular responses by the methods described above, there are alterations during a period of several hours in the ability of the vasomotor system to respond to stimulation. These alterations may result from a temporary improvement of conditions in the tissues, as indicated by the increasing CO_2 capacity, followed by a moribund condition which probably results from the abnormal circulatory and respiratory conditions, as indicated by the later fall in CO_2 capacity. We prefer to interpret the augmentation in these terms rather than in terms of a sensitization of the autonomic nervous system by hormones or other substances. We wish also to point out that the blood pressure method for the physiological assay of adrenalin is applicable only during a very limited period after the animal has been prepared. We feel that in spite of rigorous control the method is liable to be inaccurate.

SUMMARY

1. In cats which had been etherized and pithed, stunned and pithed without an anesthetic, or anesthetized with urethane, successive vasomotor stimuli of equal values, given over a period of several hours, evoked a series of vascular responses which showed a progressive augmentation (averaging 100 per cent of the initial responses) beginning about two hours after pithing, reaching a maximum in about three hours and disappearing in from three to four hours.

2. The augmentation occurred after the adrenals and thyroids had been extirpated. It occurred in animals which had been pithed through the brain to various levels of the cord, even to complete destruction. It appeared in cases in which there was a fairly constant level of blood pressure as low as 30 mm. of mercury on the one hand and as high as 130 mm. on the other. The ventilation was kept constant by artificial respiration. Ether anesthetization before pithing delayed the appearance of the augmentation, but was apparently not its cause.

3. The onset of the augmentation was correlated with an increase of the CO_2 capacity of the blood which continued after the augmentation had begun to disappear. The CO_2 capacity later decreased to a low level. Thus there appeared to be an optimum CO_2 capacity for vasomotor activity. When the CO_2 capacity was kept low by the injection of HCl an augmentation did not occur. When the CO_2 capacity was increased by the injection of sodium bicarbonate an augmentation appeared either

earlier or later than usual. In cases where an augmentation did not occur the blood pressure was low and falling and the CO_2 capacity either fell or remained at a level below that at which an augmentation appeared in other experiments.

4. The heart rate showed no appreciable alteration which could be correlated with the augmentation of the pressor responses or with the changes in CO_2 capacity. This indicates that there was no general sensitization of the myoneural junctions of the thoracico-lumbar division of the autonomic nervous system.

5. Under the conditions which exist in a study of vascular responses by the methods described above, the alterations in the ability of the vasomotor system to respond probably result from a temporary improvement of conditions in the tissues followed by a moribund condition due to the abnormal vascular and respiratory conditions. The augmentation is interpreted in these terms rather than in terms of a sensitization of the autonomic nervous system.

6. The unreliability of the blood pressure method for the physiological assay of adrenalin is pointed out.

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THE EFFECT OF COMPULSORY WORK DURING GESTATION ON THE MOTHER AND YOUNG[1]

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Received for publication February 24, 1925

In former papers (1), (2) we showed that during sexual life the normal female albino rat showed marked rhythmical fluctuations in voluntary activity, the high points coinciding with oestrus. The peaks of activity occurred approximately each fourth day during early life and were separated by as much as five days as the animal approached the menopause. It was also shown that during the period of greatest activity the female accepted the male most readily and successful copulation resulted. Though many instances were observed in which typical oestrual peaks occurred during gestation and lactation, the general rule was that they were suppressed until about the twentieth day of lactation. Oestrus and the characteristic peaks of activity were usually reëstablished about the twentieth day of lactation regardless of continued nursing of the young for a longer period. During the period of gestation and lactation the animals exhibited a marked reduction in average daily voluntary activity when compared to that shown previous to mating. This drop in activity ranged from an average daily run of 8000 or 10,000 revolutions prior to mating to approximately 3400 average daily revolutions during gestation and the first twenty days of lactation. After conception there was also an increase in the average daily food consumption during gestation and lactation. This relatively small amount of voluntary activity was characteristic of all pregnant females. Since many beasts of burden are forced to work during gestation and many classes of human beings, from force of circumstances, have to continue strenuous labor after conception it was hoped to throw some light on the effect on the mother and the offspring that this experiment was performed.

In these experiments the pregnant females were compelled to run a stipulated number of revolutions daily which represented a greatly increased amount of work as compared with that which they voluntarily performed. In no case was the number of revolutions which they were

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and The Committee for Research on Sex Problems of the National Research Council.

compelled to run equal to that voluntarily performed prior to conception. In this way we were able to determine the effect of increased work during gestation on the length of the gestation period, on the size and number of the young, and on the gain or loss of weight and the general health of the mother.

Our equipment consisted of eight revolving cages (previously described, (3)) each provided with a Veedor set-back counter. Each cage was the home of a single female. These cages were divided into two groups of four each. In one group, nos. 1 to 4, the pregnant females were allowed full freedom to run as much as they wished. These served as controls. In the other group, nos. 5 to 8, the nest boxes were removed during forced activity and the cages turned automatically. In this way pregnant females were compelled to run any number of revolutions that were desired. These cages were driven by an electric motor and an endless belt which passed in turn over each of the four cages. All these cages were therefore turned at an equal rate and for the same time interval. A variable speed motor and cone pulleys enabled us to drive the cages at from ten to twenty or more revolutions per minute. In order that these animals might have ample time for rest an apparatus driven by a weight turned an eccentric wheel, each revolution of which closed and opened, by means of a mercury key, the electric circuit to the motor. The speed of this revolving eccentric wheel was controlled by different sized revolving fans. The apparatus was so adjusted that the animals were forced to work one-third of the time and allowed to rest two-thirds of the period. The speed which we found most satisfactory was one minute of work, during which the animals were forced to run approximately twenty revolutions (110 feet), followed by two minutes of rest. This rhythm was continued for any period of time desired.

At the beginning of each day of forced work each counter was read and the number of revolutions recorded. This gave the total run during the past twenty-four hours for each rat. The counters were then set back to zero. The amount of food eaten by each rat for the past twenty-four hours was determined and recorded. Each rat was weighed and weights recorded. The nest boxes in the work cages were then removed, the belt adjusted, and the forced work begun. This was continued for different lengths of time varying from two to six or seven hours. During this period the control rats were free to sleep or run as they chose. The whole apparatus was wholly automatic and required no attention except at the starting and stopping of the forced run. At the end of the period of forced activity each counter was again read and recorded, but not reset, each rat again weighed, the nest boxes replaced and the belt removed from the cages. From this time on during the remainder of the twenty-four hours each rat was free to turn the cage ad libitum.

From these data we were able to get the average run of the controls during the work period and for the twenty-four hours, and the amount of voluntary running exhibited by the worked rats during the remainder of the twenty-four hours. We were also able to obtain the daily increase or decrease in body weight, as the embryos increased in size or were resorbed, and the amount of energy transformation, as indicated by the loss of weight during the period of work, exhibited by the two groups of rats.

Several groups of animals were tested, each test being designated as experiment 1, 2, etc. In each case the rats were allowed time to become completely accustomed to the revolving cages. As soon as all the rats showed regular cycles in their activity they were bred, the exact hour and date being recorded. In most cases all of the eight individuals were bred within four days. The beginning of the forced activity varied in different experiments from the day of mating to several days later. The first day's work was relatively small; the second day the amount was increased; usually by the third day the maximum run was given. The maximum run was continued daily until within two or three days of the time for normal delivery. After this time the animals were left undisturbed, except for daily weighings.

The work rats soon showed a tendency to hold back rather than run freely as the cage was turned. This was especially manifested toward the end of each work period and resulted in more or less sliding on "all fours." This was hard on the feet which soon became sore. To prevent this as far as possible a mixture of pine tar and tannic acid was applied, in a warm condition, to the soles of the feet. This was not only healing but served also as a protection and tended to harden the skin.

The ages of the rats of a given experiment were usually nearly the same. But in the different experiments performed a wide range in age was used. In this manner we hoped to ascertain if the age of the animal had any determining influence on the results obtained.

The food given in all the experiments was our standard diet and consisted of the following mixture by weight: ground whole wheat 3375; whole milk powder 500; commercial casein, ground fine 750; sodium chloride 50; calcium carbonate 75; sifted ground alfalfa 150; butter fat 250. The dry ingredients were first mixed and thoroughly stirred. The butter fat was then added and the mixture stirred until all the lumps were reduced to a small size. The whole mass was then put through a grinding mill which reduced it to a uniform consistency. The food was then thoroughly stirred and mixed. By this procedure we felt sure that all the ingredients were uniformly distributed throughout the mass.

From our past experiments we are confident that this diet is well suited to the albino rat and contains all the food factors necessary to promote rapid growth, to maintain normal health, and to stimulate reproduction,

lactation and raising of the young. Several litters as large as sixteen have been born and in most cases raised. The weight of the young at the age of twenty days, when they were usually weaned, was generally between 30 and 45 grams. Weights of young below 30 grams at twenty days of age were unusual and could generally be explained by some abnormal condition. Many females have exceeded a weight of 300 grams at maturity and thirteen males, picked at random, averaged 310 grams at 290 days of age. These figures are all considerably greater than those given in Donaldson's tables (5). We feel sure, therefore, that the food constituted a balanced diet. Since the food was always the same and given in amounts more than was consumed we feel sure that this factor of environment was constant.

The experiment was carried on in an isolated attic room which was in semi-darkness during the day. The temperature was almost constant, fluctuating only about 5°C. each day. The only difference in environment was that the exercised group was forced to run for a given period at definite times after which full freedom was permitted, while the control group was allowed full freedom at all times. The differences in results must therefore be due to the one environmental difference, i.e., to forced exercise.

The first two experiments were carried on jointly by the authors. In the remaining experiments Mr. Robertson was unable to assist.

Experiment 1. This experiment was started October 16, 1923, and was terminated January 6, 1924. Owing to the fact that many adjustments and changes in the apparatus were necessitated, this experiment was much delayed. The animals used were nearly the same age, all having been born in March and were approximately eight months old. No. 8 was the oldest, having been born March 1. All the others were born between the sixteenth and the twenty-eighth of March. After all the animals had become accustomed to their cages and were showing typical oestrual peaks in their activity curves, they were mated. This was accomplished by placing each female with a good breeding male in a stationary cage and allowing it to remain from December 3 to December 13. As shown later, successful matings occurred in all except rat 3. The females were returned to their respective revolving cages December 13 and the readings of the voluntary activity were taken daily. All the rats, except no. 3, showed the typical curve of gestation. Five days after being returned to the revolving cages, December 19, forced work was begun with rats 5 to 8.

The results of this experiment are briefly summarized in tables 1, 2 and 3. The exact time of mating was not observed in these cases. We have assumed that it occurred twenty-two days before delivery. These rats were forced to run but five days during the gestation period. In all cases this forced exercise was given during the daytime which is the normal

TABLE 1

Giving the total number of revolutions run by each of the control rats of experiment 1 (nos. 1 to 4), during different days of gestation, their voluntary run during the periods of compulsory work of exercised rats (table 2), and the number of young and when they were born

DATE	RAT 1				RAT 2				RAT 3				RAT 4			
	Day of gestation	Voluntary run during time of forced work	Total run for 24 hours	Young born	Day of gestation	Voluntary run during time of forced work	Total run for 24 hours	Young born	Day of gestation	Voluntary run during time of forced work	Total run for 24 hours	Young born	Day of gestation	Voluntary run during time of forced work	Total run for 24 hours	Young born
December 13	7		1,909		8		7,308				8,601		6		2,898	
December 14	8		639		9		2,342				1,312		7		140	
December 15	9		1,605		10		4,587				7,173		8		70	
December 16	10		497		11		5,929				16,085		9		179	
December 17	11		461		12		5,327				6,347		10		252	
December 18	12		1,250		13		4,687				10,035		11		358	
December 19	13	209	1,261		14	570	6,812			286	10,679		12	14	481	
December 20	14	291	528		15	341	4,832			239	17,970		13	32	620	
December 21	15	0	960		16	0	2,058			0	12,568		14	0	516	
December 22	16	84	1,104		17	186	3,612			548	22,831		15	125	556	
December 23	17		1,430		18		4,546				18,345		16		372	
December 24	18	89	1,628		19	185	3,544			481	11,590		17	28	408	
December 25	19		2,541		20		4,075				15,248		18		1,110	
December 26	20		1,428		21		3,497				21,080		19		620	
December 27	21		1,436		22		675	10			10,200		20		64	
December 28	22		1,035	5			51				9,874		21		306	
December 29			554				334				10,972		22		703	2
December 30			990				2,596				23,863				880	
December 31			1,377				1,920				13,062				500	
January 1			1,318				7,270				11,944				783	
January 2			1,216				6,544				18,521				229	
Daily average		135	1,198			256	3,931			311	13,252			40	574	

TABLE 2

Giving the total number of revolutions run by each of the exercised rats of experiment 1, nos. 5 to 8, during different days of gestation, the number of revolutions of compulsory work, and the number of young and when they were born

DATE	RAT 5				RAT 6				RAT 7				RAT 8			
	Day of gestation	Forced work	Total run for 24 hours	Young born	Day of gestation	Forced work	Total run for 24 hours	Young born	Day of gestation	Forced work	Total run for 24 hours	Young born	Day of gestation	Forced work	Total run for 24 hours	Young born
December 13	7		55		7		0		6		307		10		23	
December 14	8		24		8		247		7		974		11		527	
December 15	9		385		9		1,951		8		3,062		12		1,052	
December 16	10		824		10		1,597		9		3,058		13		877	
December 17	11		1,543		11		2,616		10		3,742		14		1,727	
December 18	12		1,918		12		4,868		11		7,507		15		3,829	
December 19	13	1,922	4,311		13	2,006	5,412		12	1,873	6,356		16	1,809	3,212	
December 20	14	2,400	2,444		14	2,460	4,958		13	2,293	3,328		17	2,350	3,011	
December 21	15	1,148	1,296		15	1,248	2,867		14	1,231	3,082		18	1,225	1,285	
December 22	16	2,295	2,460		16	2,396	4,846		15	2,348	9,160		19	2,260	2,288	
December 23	17		83		17		1,985		16		9,346		20		10	
December 24	18	2,541	4,784		18	2,464	7,354		17	2,653	9,939		21	2,613	4,052	D
December 25	19		2,152		19		273		18		13,401					
December 26	20		2,015		20		909		19		11,858					
December 27	21		1,226		21		302		20		5,782					
December 28	22		583	6	22		329	6	21		4,602					
December 29			1,022				1,700		22		1,091	8				
December 30			5,708				1,390				10,585					
December 31			1,482				2,653				9,095					
January 1			4,229				8,147				10,694					
January 2			3,517				9,277				13,492					
Daily average		2,061	2,003			2,115	3,032			2,080	6,693			2,051	1,828	

sleeping time for the rat. The forced work was started about 9 a.m. each day and continued without change or interruption for the entire exercise period. The amount of forced exercise during each of these five days is shown in table 2. A comparative summary of the results of control and exercised rats during the days of forced activity is given in table 3. As will be noticed from these tables, the first four days of forced work were consecutive and that one day intervened before the fifth day of forced work. This was necessitated because of sore feet. It will also be noted that the amount of forced work given during each of these days was far in excess of that performed by the control rats. It was more than fourteen times as great. But notwithstanding this amount of forced work the exercised animals voluntarily ran during the night time practically as

TABLE 3

Summary of tables 1 and 2, comparing the results of the pregnant control and exercised rats of experiment 1, during the five days of compulsory work

DATE	CONTROL RATS			EXERCISED RATS							
				Forced work							
				Time in minutes							
	Average voluntary run during period of forced work	Average voluntary run for remainder of 24 hours	Average total voluntary run for 24 hours	Total period	At work	Average total revolutions	Revolutions per minute of work	Average run for remainder of 24 hours	Average total run for 24 hours	Number of revolutions in excess of controls	Ratio of total activity of control to exercised rats
December 19	262	2,703	2,965	375	125	1,903	15.2	2,920	4,823	1,858	61:100
December 20	221	1,772	1,993	420	140	2,376	17.0	1,059	3,435	1,442	58:100
December 21	0	1,178	1,178	240	80	1,213	15.1	919	2,132	974	55:100
December 22	132	1,625	1,757	315	105	2,325	22.1	2,365	4,690	2,933	82:100
December 24	101	1,759	1,860	240	80	2,568	32.1	3,964	6,532	4,672	40:100
Average	143	1,807	1,951	318	106	2,075	20.3	2,245	4,322	2,376	45:100

much, and in some cases more than the controls. The time elapsing between the end of the forced work period and the normal activity period was apparently sufficient for complete recovery. The ratios of activity of the two groups are given in table 3.

A comparison of the voluntary activity of these two groups of animals during the days in which no forced work was given shows that the controls were slightly less active. The control rats averaged 2018 revolutions of voluntary activity daily while the other group averaged 2486 revolutions. Almost the same difference is seen in the average daily voluntary run of the two groups during the days of forced work (table 3). We must therefore conclude that the amount of forced work given during the day time did not

apparently interfere with the normal voluntary activity during their usual period of activity.

Table 3 also shows that the rate of running was increased on succeeding days. During the last work period the number of revolutions per minute was more than twice that on the first day. This enabled us to shorten the exercise period and yet give a greater amount of work. We were thus able on the last day to compel the rats to run over a half-mile farther than the first day in two hours and fifteen minutes less time. The voluntary run following this period, however, was greater than on any of the other days. This indicates that the rat is able to perform more work in a shorter time more easily than less work in a longer time. At the end of the fourth hour of the fifth day of forced exercise rat 8 was found dead. All the others were in fair condition. Autopsy showed the presence of three young which would have been born the next day. The uteri were badly congested and showed two resorption areas. There were six distinct corpora lutea of gestation and a seventh questionable one. All other organs appeared normal.

No further forced exercise was given during this experiment. The remaining pregnant rats delivered young, as indicated in tables 1 and 2.

This experiment seems to indicate that pregnant rats can perform a certain amount of work in addition to their voluntary nightly run if the work is not too great and if it be given during the daytime, provided that it be given not later than the seventeenth day of gestation. If given as late as the twentieth day it may result in the death of the mother.

This experiment also illustrates a fact which we emphasized in a former paper (2). That is, it is a tendency for rats carrying small litters to be less active than those carrying large litters. Rat 4, which had a litter of two young, had a daily average of 574 revolutions. Rat 2, which gave birth to ten young, averaged 3931 revolutions daily. Rat 1 was intermediate both in the number of young and in activity. Rats 6, 7 and 8, of the exercised group, also show the same conditions.

Experiment 2. This experiment extended from January 9 to March 2, 1924. All of the animals used, except no. 1, were born October 22, 1923 and were seventy-nine days old at the beginning of the experiment. The exact date of birth of no. 1 was not known, but it was approximately one hundred days old. Within two or three weeks all were showing typical oestrual cycles in their activity curves. As in the previous experiment, nos. 1, 2, 3 and 4 were used as controls and 5, 6, 7 and 8 were given forced exercise. Attempts at mating were made from the tenth to the thirteenth of February. Forced exercise was begun February 14 and with the exception of February 16 and 17 was continued daily until March 2. The results of this experiment are shown in tables 4, 5 and 6.

The curves of activity of our control rats soon showed that none of them

were pregnant. The rhythmic increase and decrease in the number of daily revolutions voluntarily turned illustrates this (table 4). We have no means of ascertaining whether our exercised rats were pregnant. If they were the forced work caused complete resorption as shown by the autopsies which will be given later.

A marked difference is noticed in the average total daily activity of the non-pregnant control rats and the exercised rats. The control rats

TABLE 4

Giving the voluntary run of control rats during the period of forced exercise in experiment 2

DATE	RAT 1		RAT 2		RAT 3		RAT 4		AVERAGE VOLUNTARY RUN FOR 24 HOURS
	Day after mating	Voluntary run for 24 hours	Day after mating	Voluntary run for 24 hours	Day after mating	Voluntary run for 24 hours	Day after mating	Voluntary run for 24 hours	
February 14	4	14,682	2	14,219	4	21,960	4	3,787	13,637
February 15	5	6,962	3	12,500	5	9,904	5	10,498	9,966
February 16	6	9,173	4	13,023	6	16,716	6	17,465	14,094
February 17	7	14,682	5	22,148	7	19,422	7	9,382	16,409
February 18	8	20,291	6	10,756	8	26,573	8	9,173	16,698
February 19	9	9,405	7	8,815	9	12,955	9	17,379	12,139
February 20	10	13,224	8	17,383	10	23,278	10	22,970	19,214
February 21	11	14,546	9	20,883	11	19,192	11	10,899	16,380
February 22	12	16,996	10	11,593	12	29,391	12	10,422	17,100
February 23	13	7,341	11	10,817	13	16,225	13	16,535	12,729
February 24	14	6,715	12	16,512	14	15,762	14	21,212	15,050
February 25	15	16,169	13	23,413	15	21,907	15	11,451	18,235
February 26	16	15,325	14	8,776	16	31,055	16	23,204	19,590
February 27	17	9,185	15	14,575	17	15,031	17	9,610	12,100
February 28	18	10,788	16	16,484	18	15,490	18	22,347	16,277
February 29	19	13,858	17	21,386	19	20,819	19	11,695	16,937
March 1	20	18,742	18	5,095	20	30,457	20	13,018	19,328
March 2	21	7,018	19	7,894	21	20,873	21	18,177	13,490
Daily average		12,505		14,237		20,389		14,396	15,384

averaged 15,384 revolutions daily while the worked rats ran only an average of 6313 revolutions. This last number includes not only the voluntary run, but also the forced work. Since none of the rats in this experiment gave birth to young we are unable to state whether this great difference was due to early pregnancy and resorption in the exercised rats or to the forced work. Later experiments were planned to throw some light on this.

TABLE 5

Giving the amount of forced work, voluntary and total revolutions, and relation of controls to exercised rats in experiment 2

DATE	RAT 5			RAT 6			RAT 7			RAT 8			FORCED WORK	AVERAGE VOLUNTARY RUN FOR REMAINING 24 HOURS	AVERAGE TOTAL RUN FOR 24 HOURS	RATIO TOTAL AVERAGE ACTIVITY OF CONTROL TO EXERCISED RATS
	Day after mating	Voluntary run for remaining 24 hours	Total run for 24 hours	Day after mating	Voluntary run for remaining 24 hours	Total run for 24 hours	Day after mating	Voluntary run for remaining 24 hours	Total run for 24 hours	Day after mating	Voluntary run for remaining 24 hours	Total run for 24 hours				
February 14	4	285	912	4	515	1,242	4	734	1,361	4	388	1,015	627	480	1,142	1,250:100
February 15	5	6,695	8,427	5	11,880	13,512	5	3,492	5,225	5	2,140	3,872	1,732	6,052	7,759	131:100
February 16	6		18,092	6		20,123	6		8,723	6		7,226		13,541	13,541	104:100
February 17	7		7,962	7		4,488	7		3,871	7		3,153		4,868	4,868	337:100
February 18	8	3,547	6,864	8	1,340	4,657	8	1,328	4,657	8	358	3,675	3,317	1,643	4,960	337:100
February 19	9	5,726	9,698	9	6,303	10,275	9	1,233	5,205	9	320	4,292	3,972	3,395	7,368	164:100
February 20	10	4,359	8,718	10	3,211	7,570	10		1,779	10	98	4,457	4,359	2,556	5,629	343:100
February 21	11	3,739	6,300	11	2,151	4,712	11		*	11	829	3,390	2,561	2,239	4,880	342:100
February 22	12	2,282	5,423	12	2,019	5,160	12			12	98	3,239	3,141	1,466	4,607	372:100
February 23	13	374	3,016	13	8,303	10,945	13			13	1,198	3,840	2,642	3,292	5,933	215:100
February 24	14	5,406	7,487	14	5,803	7,884	14			14	3,868	5,767	2,081	4,965	7,046	214:100
February 25	15	819	3,280	15	839	3,300	15	6,842	9,203	15	1,279	3,740	2,461	2,444	4,880	372:100
February 26	16	258	2,371	16	2,406	4,519	16	2,838	4,951	16	5,628	6,741	2,113	2,782	4,646	416:100
February 27	17	1,428	5,423	17	7,407	11,402	17	6,008	10,013	17	5,907	9,902	3,995	5,187	9,185	134:100
February 28	18	2,097	5,658	18	1,293	4,854	18	652	4,213	18	648	4,209	3,561	1,172	4,733	346:100
February 29	19	12,134	15,254	19	2,126	5,246	19	154	3,274	19	5,737	8,857	3,120	5,037	8,157	209:100
March 1	20	3,259	5,680	20	10,382	12,803	20	2,865	5,286	20	5,622	9,043	3,421	5,532	8,203	235:100
March 2	21	1,597	4,121	21	11,049	13,573	21		159	21	4,366	6,890	2,529	4,254	6,186	218:100
Daily average		3,375	6,924		5,647	8,126		1,294	4,851		2,704	5,184	2,789	3,939	6,313	318:100

* Counter not working.

If we compare the activity of the rats in this experiment with that of experiment 1 we also see a great difference. The average daily activity of the non-pregnant rat (no. 3) of experiment 1 compares very favorably with the average daily activity of the non-pregnant controls of this experiment. But if the pregnant controls of the first experiment are compared with the non-pregnant controls of this experiment we find that the latter ran eight times as many revolutions as the former. When the exercised rats of the two experiments are compared we find that the rats of this

TABLE 6

Showing the weight of each rat at different intervals during experiment 2

DATE	CONTROL RATS				EXERCISED RATS			
	1	2	3	4	5	6	7	8
January 9	150	102	105	140	118	116	112	89
January 12	157	122	117	147	123	126	132	98
January 15	163	128	130	158	133	136	138	108
January 18	159	122	130	160	130	130	138	106
January 22	161	120	126	149	132	132	138	104
January 25	170	137	148	182	146	146	148	130
January 27	162	140	149	173	148	146	146	128
January 29	167	150	158	187	158	154	154	144
February 1	170	158	171	196	152	152	152	145
February 5	169	165	175	196	160	155	160	149
February 9	172	170	178	195	166	158	168	156
February 13	170	182	196	198	176	148	168	159
February 17	171	180	194	200	178	159	174	163
February 20	175	180	193	210	183	172	181	167
February 25	182	184	199	197	200	170	176	165
February 29	189	190	214	218	192	164	163	163
March 2	178	184	194	202	190	150	160	150
Gain	28	82	89	62	72	34	48	61
Average gain	65				54			

experiment ran almost two times as much as the exercised rats of experiment 1 which delivered young. This indicates that if conception had occurred in the exercised rats resorption took place at an early date.

The weights of the different individuals, as shown in table 6, do not indicate pregnancy. They conform closely to normal growth. The difference in average gain in weight of the two groups is also significant. The control rats, which averaged to run almost nine and one-half miles each day more than the exercised rats, had an average gain of eleven grams greater than the exercised group. This seems to indicate that forced work of the

type given required a much greater expenditure of energy than voluntary work. Though during the forced work two-thirds of the time was allowed for rest, there was no chance for a longer period of rest. The custom of holding back and trying to keep from running as the cage turned no doubt required a much greater expenditure of energy per revolution than was used in a revolution of voluntary running. In later experiments we have endeavored to indicate this energy expenditure by the loss in weight of the animal.

At the end of this experiment all the exercised rats were killed and autopsies made, in order to determine if possible whether conception and resorption had occurred. Rat 5 showed the uteri quite congested and swollen. There was no sign of embryos nor any foci of resorption. There were four distinct corpora lutea present. All other organs were normal. Evidently this rat had either been pregnant and complete resorption had occurred, but not sufficient time had elapsed to allow a complete clearing up of the uteri, or the swollen and congested condition of the uteri may have been due to normal changes which occur during stages one and two of oestrus. The relatively large run of this rat on February 29 rather indicates that the congested condition was due to normal oestrus. Rats 6, 7 and 8 were found normal in regard to all organs. The uteri were in a state similar to dioestrum. If conception had taken place in any of these rats sufficient time had passed to permit complete resorption and return of all the reproductive organs to a normal condition. The total daily run of each of these animals throughout the experiment strongly suggests that none of them were pregnant.

The most important fact indicated by this experiment is that compulsory exercise apparently causes a greater expenditure of energy per revolution than voluntary running. A direct comparison of the number of revolutions turned as indicating the amount of work performed is, therefore, not justifiable.

Experiment 3. This experiment was started March 8 and concluded May 22, 1924. At the beginning of the experiment the animals used had the following ages and weights:

	CONTROL RATS				EXERCISED RATS			
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
Age in days	168	138	69	138	138	69	77	77
Weight in grams	174	205	108	208	197	114	126	131

With the exception of rat 4, successful mating occurred in all the animals between April 16 and April 29. The total daily voluntary runs of rat 4, as shown in table 7, indicate that pseudopregnancy had resulted from the

numerous mating trials and that regular oestrus was not reëstablished until May 5. The days of successful mating of the other females are indicated in tables 7 and 8.

The forced work was started April 22 and continued as shown in tables 7 and 8 until May 12. The length of each forced activity period, and the exact amount of time during this period the animals were forced to run are given in table 9. This table also gives the average weight of the exercised rats at the beginning of each forced work period and the average loss in weight they sustained during the period. In this way we hoped to establish a relation between the energy turnover, as measured by loss of weight, and the amount of forced work given. Unfortunately in this experiment only a few similar weighings were taken of our controls.

With the exception of April 22 and 25, the time of the day at which the forced exercise was given in this experiment was in the late afternoon or in the evening. Since the rat is normally a nocturnal animal, this was at a time when they were usually just beginning their normal voluntary activity. On April 22 and 25 the forced work was started at 10 a.m. and stopped at 3 and 4 o'clock p.m., respectively. On each of these two days the average voluntary run for the remaining twenty-four hours was far in excess of any day in which the compulsory work was given in the late afternoon or evening (tables 8 and 9). This corresponds to the results as given in experiment 1, in which all the forced activity was given during the middle of the day. This arrangement apparently allowed sufficient time for recovery before the normal period of activity. When the forced work was given in the evening it usurped a part of the period in which the rats were normally active and fatigued the animals to such an extent that, with no time for rest, the following voluntary run was greatly reduced. This is especially emphasized by comparing the average ratios of total activity of control to exercised rats in experiment 1, table 3, and this experiment, table 9. In the first case the average ratio of controls to exercised rats was 45:100 while in the last case it was 180:100, respectively. The conditions of these two experiments were very similar with the exception of the time of day at which the compulsory work was given. That is, all the animals compared were pregnant, were confined in the same cages, in the same room, fed the same food and exercised in a similar manner, but at different times of the day. Experiment 2, table 5, also shows a similar relation which existed between non-pregnant control and exercised rats which were forced to run in the evening.

In the two former experiments the food consumption was not ascertained. In this experiment the food boxes were provided with removable hoods (previously described (2)) which prevented wasting of food and permitted us to determine the daily food intake. Table 9 shows that the average daily food consumed by the pregnant control rats was 20.4 grams

and that for the exercised rats was 18.6 grams. This forms a ratio between the control and exercised rats of approximately 110 to 100. If we consider the average daily food consumption and total daily run of the control and exercised groups we find that one gram of food was equivalent to approximately 179 and 109 revolutions respectively. This again indicates that there is more energy expended in one revolution of forced work than in a revolution voluntarily turned, the ratio of expenditure being 164 to 100, respectively. Later experiments have shown that the rate of increase in weight of the mother and the number of young that are being developed must also be taken into consideration.

During the periods of forced exercise there was an average loss of weight in the exercised rats of 15.6 grams per period. The loss of weight in the control rats during these periods was not determined in a sufficient number of cases to warrant a comparison. While some of the loss in weight was due to the excretion of urine and feces, which we were unable to measure, a large part represented the energy expended in the work performed. Table 9 shows that in general the longer periods of forced work were accompanied with greater losses in body weight. A greater number of observations would eliminate the variations that are noted. In other experiments we have found that the loss of weight of an average rat, due to resting metabolism, is from 0.5 to 0.6 gram per hour. In the very short resting periods of our exercised rats it would doubtless be greater. In the average forced work period of 210 minutes (table 9) during which an average of 15.6 grams in body weight was lost, approximately 2 or 2½ grams would be due to resting metabolism. This would leave approximately 13 grams equal to 1337 revolutions, or one gram equaled about 103 turns of forced work.

The data of this experiment are too meager to warrant any definite conclusions relative to the effect of the number of young being developed to the loss of weight of the mother during forced work. It is interesting to note, however, that rats 5 and 7, which gave birth to eight and twelve young respectively on the same day, had an average loss of 12.3 and 10.9 grams respectively. These two rats had almost the same daily food consumption.

In this experiment rats 2 and 6 ate their young at birth. Tables 7 and 8 show the effect of this on the weighed food consumption. This indicates that the litter of rat 2 was considerably larger than that of rat 6. A loss of weight of the mother of 45 grams at delivery also suggests a rather large litter.

Rats 1 and 3 of the controls, and rats 5 and 7 of the exercised group all had litters which were raised. The average size of the litters in the control group was 8.5 while that of the exercised group was 10. This seems to indicate that the amount of forced exercise given these rats had little effect

TABLE 7

Giving the day of mating of control rats (C), their voluntary run during the period of forced work of exercised rats, their total run for each twenty-four hours, and the number of young and the day they were born (Y). Experiment 3

DATE	RAT 1				RAT 2				RAT 3				RAT 4				AVERAGE RUN FOR 24 HOURS OF 1, 2 AND 3	AVERAGE VOLUNTARY RUN DURING PERIOD FORCED WORK OF 1, 2 AND 3		AVERAGE FOOD EATEN (1, 2 AND 3)
	Day after mating	Voluntary run during period forced work	Total run for 24 hours	Grams food eaten	Day after mating	Voluntary run during period forced work	Total run for 24 hours	Grams food eaten	Day after mating	Voluntary run during period forced work	Total run for 24 hours	Grams food eaten	Day after mating	Voluntary run during period forced work	Total run for 24 hours	Grams food eaten			AVERAGE VOLUNTARY RUN REMAINDER 24 HOURS OF 1, 2 AND 3	
April 22			6,763	20			6,898	21			1,825	22			5,477	9	5,162			21
April 23			9,893	18			8,291	20			3,257	12			6,759	12	7,147			16.7
April 24	C		13,076	28			11,531	20			6,138	18			7,661	16	10,248			22
April 25	1	0	293	17	C	1,283	20,386	21		717	9,720	16		300	5,680	19	10,133	667	9,466	18
April 26	2	372	11,133	23	1	980	3,091	21		11	4,398	18		1,075	2,714	17	6,207	454	5,753	20.7
April 27	3	1,383	2,725	19	2	120	5,255	16		212	8,273	20		733	6,545	14	5,418	572	4,846	18.3
April 28	4		3,127	19	3		4,145	12			4,154	18			2,077	24	3,809			16.3
April 29	5		507	23	4		694	28	C		12,543	18			1,289	24	4,581			23
April 30	6	406	931	19	5	667	1,836	16	1	1,273	2,151	16		1,339	1,922	18	1,639	782	857	17
May 1	7	106	1,496	20	6	711	2,799	23	2	830	7,920	18		13	2,781	19	4,072	549	3,523	20.3
May 2	8	0	449	17	7	64	1,114	25	3	17	3,921	19		4	1,431	20	1,828	27	1,801	20.3
May 3	9	0	372	23	8	182	991	31	4	1,183	6,986	22		148	666	24	2,783	455	2,328	25.3
May 4	10	0	957	19	9	0	870	20	5	3	4,865	15		0	1,325	16	2,231	1	2,230	18
May 5	11	28	711	18	10	19	2,010	25	6	150	4,519	13		1,032	12,120	12	2,413	66	2,347	18.7
May 6	12	0	411	28	11	166	1,925	28	7	18	3,421	25		72	407	22	1,919	61	1,858	27
May 7	13	0	506	24	12	1,080	1,627	24	8	0	4,535	20		0	5,193	18	2,223	360	1,863	22.7

May 8	14	436	595	26	13	508	3,603	17	9	781	7,783	21		213	13,465	14	3,987	908	3,079	21.3
May 9	15	3	597	20	14	147	1,398	25	10	165	4,579	17		23	11,234	13	2,191	516	1,675	20.7
May 10	16	1	1,463	27	15	87	1,834	23	11	25	6,858	25		0	5,966	24	3,385	38	3,347	25
May 11	17	0	1,135	18	16	27	2,668	21	12	18	6,457	16		18	1,384	9	3,420	15	3,405	18.3
May 12	18	39	1,625	17	17	259	2,238	23	13	31	4,928	20		555	9,944	16	2,930	110	2,820	20
May 13	19		1,201	25	18		836	28	14		6,854	24			18,022	9	2,899			23.7
May 14	20		568	20	19		1,771	21	15		5,460	24			7,790	14	2,600			21.7
May 15	21	8Y	872	21	20		956	28	16		2,792	22			6,890	15	1,540			23.7
May 16			236	14	21		1,209	18	17		5,753	24			15,769	15	2,399			18.7
May 17			232	16	22	Y-E	1,924	20	18		1,982	25			16,855	13	1,379			20.3
May 18			554	23			11	2	19		2,892	25			3,996	16	1,152			16.7
May 19			361	23			66	10	20		998	22			2,529	16	475			18.3
May 20			304	25			238	12	21	9Y	2,083	15			11,786	15	875			17.3
May 21			86	25			932	9			820	26			12,795	15	613			20
May 22			78	25			3,759	5			463	15			6,662	14	1,433			15
Daily average		173	1,191	21.3		400	1,885	19.7		512	4,280	19.7		346	6,748	16 2	3,325	349	3,199	20 2

TABLE 8

Giving the day of mating of exercised rats (C), the amount of forced run, the total run for each twenty-four hours, and the number of young and when they were born (Y), experiment 3

DATE	RAT 5				RAT 6				RAT 7				RAT 8				AVERAGE RUN FOR 24 HOURS	AVERAGE FORCED RUN	AVERAGE VOLUNTARY RUN FOR REMAINDER 24 HOURS	AVERAGE FOOD EATEN
	Day after mating	Forced rnn	Total for 24 hours	Grams food eaten	Day after mating	Forced run	Total for 24 hours	Grams food eaten	Day after mating	Forced run	Total for 24 hours	Grams food eaten	Day after mating	Forced run	Total for 24 hours	Grams food eaten				
April 22	6		5,452		C	2,827	11,726	14	6	3,456	6,980	19	6	3,176	4,947	22	7,276	3,146	4,130	18.3
April 23	7		10,785	16	1		17	18	7		16	15	7		12	16	2,708			16.2
April 24	8		3,104	18	2		325	25	8		1,200	20	8		681	19	1,338			21.0
April 25	9	2,651	6,158	19	3	2,584	2,892	18	9	2,609	7,006	19	9	2,575	3,931	21	4,997	2,605	2,392	19.2
Aptil 26	10	973	1,914	1	4	964	985	20	10	968	1,080	20	10	959	963	7	1,236	966	270	12.0
April 27	11	1,283	1,340	23	5	1,267	1,288	19	11	1,275	1,370	16	11	1,256	1,259	12	1,314	1,270	44	17.5
April 28	12		37	22	6		24	22	12		140	23	12		47	20	62			21.8
April 29	13		550	29	7		179	27	13		3,884	25	13		159	23	1,193			26.0
April 30	14	1,639	2,023	22	8	1,637	2,089	19	14	1,643	2,255	14	14	1,626	1,912	19	2,070	1,636	434	18.5
May 1	15	2,105	3,065	21	9	2,101	2,947	16	15	2,084	2,996	17	15	2,080	2,138*	15	2,787	2,092	695	17.2
May 2	16	944	977	20	10	926	928	21	16	955	1,003	19					969	942	27	20.0
May 3	17	1,255	1,358	27	11	1,254	1,289	16	17	1,248	1,275	21					1,307	1,252	55	21.3
May 4	18	747	968	24	12	745	890	20	18	754	846	20					901	749	152	21.3
May 5	19	2,110	2,971	18	13	2,112	2,294	16	19	2,101	2,323	17					2,529	2,108	421	17.0
May 6	20	219	242	13	14	1,262	1,287	20	20	217	218	16					582	566	16	16.3
May 7	21	8Y	311	13	15	1,250	1,600	17	21	12Y	84	11					665			13.7
May 8			717	22	16	586	735	21			115	18					522			20.3
May 9			359	15	17	963	1,196	17			1,005	15					853			15.7
May 10			847	26	18	1,735	1,768	23			606	21					1,074			23.3

May 11			896	15	19	1,498	1,661	15			370	13					976			14.3
May 12			1,134	19	20	1,189	1,476	20			3,147	30					1,919			23.0
May 13			1,009	44	21	Y-E	79	9			707	16					598			23.0
May 14			1,210	23			3,202	11			2,024	23					2,145			15.7
May 15			1,066	27			3,037	12			413	38					1,505			25.7
May 16			567	30			4,489	14			744	34					1,933			26.0
May 17			1,385	29			4,436	15			1,063	44					2,295			29.3
May 18			1,389	33			5,111	18			851	45					2,450			32.0
Daily average		1,393	1,920	21.8		1,465	2,143	17.9		1,574	1,619	21.1		1,945	1,605	17.9	1,785	1,576	785	20.2

* Died during forced work.

TABLE 9

Giving the average results of tables 7 and 8, the duration of work period, time at work, average weight and loss, and the ratio of total activity of control and exercised rats in experiment 3

DATE	CONTROL RATS				EXERCISED RATS									
					Forced work									
					Time in minutes									
	Average food eaten	Average voluntary run during period of forced work	Average voluntary run for remaining 24 hours	Average total voluntary run for 24 hours	Total period	At work	Average weight at beginning	Average weight lost	Average revolutions	Revolutions per minute of work	Average food eaten	Average run for remaining 24 hours	Average total run for 24 hours	Ratio of total activity of control to exercised rats
April 22	21.0			5,162	300	100			3,146	31.4	18.3	4,130	7,276	71:100
April 25	16.7	667	9,466	10,133	360	120	214	18.5	2,605	21.7	16.2	2,392	4,997	213:100
April 26	22.0	454	5,753	6,207	180	60	195	5.25	966	16.1	21.0	270	1,236	502:100
April 27	18.0	572	4,846	5,418	180	60	208	12.2	1,270	21.2	19.2	44	1,314	413:100
April 30	20.7	782	857	1,639	405	135	224	17.2	1,636	12.1	12.0	434	2,070	82:100
May 1	18.3	549	3,523	4,072	300	100	223	15.5	2,092	20.9	17.5	695	2,787	146:100
May 2	16.3	27	1,801	1,828	180	60	223	10.3	942	15.4	21.8	27	960	189:100
May 3	23.0	455	2,328	2,783	180	60	231	10.0	1,252	20.9	26.0	55	1,307	209:100
May 4	17.0	1	2,230	2,231	135	45	239	11.0	749	16.6	18.5	152	901	247:100
May 5	20.3	66	2,347	2,413	210	70	245	15.0	2,108	30.1	17.2	421	2,529	99:100
May 6	20.3	61	1,858	1,919	120	40	239	6.7	566	14.1	20.0	16	582	329:100
May 7	25.3	360	1,863	2,223	180*	60	204	9.0	1,250	20.8	21.3	350	1,600	139:100
May 8	18.0	908	3,079	3,987	120	40	213	9.0	586	14.7	21.3	149	735	543:100
May 9	18.7	516	1,675	2,191	180	60	220	14.0	963	16.1	17.0	233	1,196	183:100
May 10	27.0	38	3,347	3,385	240	80	224	19.0	1,735	21.7	16.3	33	1,768	191:100
May 11	22.7	15	3,405	3,420	210	70	234	12.0	1,498	21.7	13.7	163	1,661	206:100
May 12	21.3	110	2,820	2,930	180	60	240	13.0	1,189	19.5	20.3	287	1,476	199:100
Daily average	20.4	349	3,199	3,644	210	70		15.6	1,337	19.7	18.6	579	2,023	180:100

* The remaining figures in this table under exercised rats are for rat 6, the only one still pregnant.

on the number of young born. The data relative to the length of the gestation periods show similar results. The average for each group was twenty-one days and six hours.

Rat 8 died as the result of compulsory exercise on her fifteenth day of gestation. On this day, which was her sixth day of forced work (see table 8), work was started at 4:30 p.m. Five hours later this rat was found rolling in the cage. It was removed from the cage and found to be breathing, but died fifteen minutes later. Autopsy showed the following organs normal: intestines, stomach (partly filled with food), kidneys, adrenals, spleen, liver, heart and lungs. The animal was in a fat condition. The uteri were congested, four embryos in left and seven in right. There was no indication of resorption. Weight of the eleven embryos, cleared from amniotic fluid, placenta, etc., was 2.8 grams. This gave an average weight of a little over 0.254 gram. This is 0.086 gram heavier than the average weight of fetuses at 15 days given by Stotsenburg (4) in Donaldson's (5) reference tables for the albino rat. All the embryos appeared normal and of uniform size. Apparently the forced work given did not retard the early development of the young in this case. Both ovaries were found normal. The left had five and the right had nine distinct corpora lutea of gestation.

This experiment seems to indicate that forced exercise given in the evening reduces the voluntary run of the rat during the remaining twenty-four hours; that one gram of food eaten during pregnancy is equivalent to the production of 179 voluntary revolutions and 109 forced revolutions, a ratio of 164 to 100; that the energy turn-over as indicated by one gram loss of weight of the rat is equal to 103 revolutions of forced work which is almost the same relation as the food consumed; that the amount of work given did not apparently influence the number of young born by the rats which endured throughout the gestation period; that death may result to pregnant females from forced work; and that the early development of the young was apparently not retarded.

Experiment 4. The animals used in this experiment were placed in their respective cages May 22, 1924. This experiment was discontinued July 21. The stock used was selected from vigorous females which had been used in an experiment on the effect on activity of gestation and lactation. They were all accustomed to turning the revolving cages which permitted an early start in forced activity. The weight and age on May 22 and a brief summary of the early history of each of these rats is given in the tabulation on the following page.

As can be seen, these animals were within five days of the same age. Those of the same age were sisters. They were as nearly related as we were able to secure at that time. All were about in their prime and represent older animals than in the preceding experiments.

* The remaining figures in this table under exercised rats are for rat 6, the only one still pregnant.

	CONTROL RATS				EXERCISED RATS			
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8
Weight in grams	274	242	282	231	223	246	234	233
Age in days	342	342	342	337	337	337	337	337
Number of previous litters	2	3	2	3	2	2	3	2
Number of young in previous litters	5	20	14	13	17	9	25	15
Days since last litter	37	50	63	62	59	61	43	28

Numerous attempts at mating were made and coitus occurred in all. But as it was afterwards proved only control rat 2 and exercised rat 6 became pregnant. The forced exercise, however, had proceeded so far that it was continued until within four days of delivery of litter by rat 6. The duration of the periods of forced exercise was from June 1 to June 19. During this time the exercised rats were given forced work daily.

Though little relative to the effect of forced exercise during gestation was obtained some very useful information was acquired in regard to the effect of compulsory exercise on non-pregnant rats. While one pregnant rat in each group is insufficient to warrant any general conclusions, if their behavior corresponds to that of the previous experiments we can make a comparison between the effect on pregnant and non-pregnant rats.

Summaries of the results of non-pregnant and pregnant rats during the same period of the experiment are given in tables 10 and 11. During the time forced work was given all the rats were weighed daily and the individual loss in weight during the work period was determined. Only the averages are given in table 10. Since only one control and one exercised rat became pregnant table 11 represents individual weights and activities. The forced exercise was given in all cases in the evening.

Table 10 shows that the non-pregnant control rats made an average voluntary run of 68 revolutions during each period of forced work while the non-pregnant exercised rats were compelled to run 1408 revolutions. This indicates that the periods of forced work stopped about the time the rats were about to begin their normal activity. The total runs for twenty-four hours of the control and exercised groups formed a ratio of 295 to 100. This corresponds to the results in the earlier experiments. Quite similar results are seen in the pregnant rats in table 11. The pregnant exercised rats, however, show a much greater voluntary run after exercise than the corresponding group of non-pregnant animals. This may have been due to individual variation.

The average daily food consumed by the non-pregnant controls was 20.7 grams and for the exercised group was 12.9 grams. This forms a ratio of 164 to 100. As would be expected, the food consumed by the pregnant rats was greater than the corresponding group of non-pregnant rats. In

TABLE 10

Summary of results of non-pregnant rats in experiment 4

DATE	CONTROL RATS					EXERCISED RATS								
						Forced work								
						Time in minutes								
	Average grams food eaten in 24 hours	Average voluntary run during period forced work	Average total run for 24 hours	Average weight at beginning forced work period	Average weight lost during period	Total period	At work	Average weight at beginning	Average weight lost	Average revolutions	Revolutions per minute	Average grams food eaten in 24 hours	Average total run for 24 hours	Ratio of total average activity of control to exercised rats
June 1	20.2	11	7,830	264	1.5	180	60	257	12.7	1,125	18.8	21.7	2,673	293:100
June 2	17.8	106	2,759	260	1.5	225	75	249	12.0	2,000	26.9	14.5	2,019	135:100
June 3	21.0	196	2,712	266	3.5	240	80	247	15.7	2,034	29.6	6.7	2,035	133:100
June 4	20.2	10	2,440	269	3.0	120	40	238	9.0	895	24.0	7.3	913	267:100
June 5	19.0	132	8,993	268	4.0	180	60	234	10.0	1,279	21.2	8.7	1,289	690:100
June 6	21.0	4	3,941	275	2.0	120	40	227	10.3	918	23.2	9.3	925	425:100
June 7	21.7	12	1,302	277	1.7	150	50	227	4.0	1,020	20.3	10.3	1,020	128:100
June 8	20.3	67	3,788	279	4.0	180	60	230	7.5	1,035	17.8	12.0	1,285	293:100
June 9	20.0	4	1,210	274	1.5	180	60	225	4.3	1,313	21.9	12.3	1,380	88:100
June 10	22.0	79	3,837	276	5.0	240	80	230	8.8	2,225	28.1	15.7	3,183	120:100
June 11	24.3	21	2,997	277	4.7	135	45	229	8.8	1,050	26.2	15.7	1,065	281:100
June 12	22.0	5	4,985	268	6.7	195	65	230	11.7	1,800	27.7	17.3	1,846	270:100
June 13	18.3	9	7,898	274	6.3	135	45	226	10.3	1,228	27.3	12.3	1,249	633:100
June 14	22.0	53	4,363	281	8.0	240	80	228	13.7	2,218	27.7	14.0	3,458	128:100
June 15	22.0	102	2,687	281	4.3	165	55	225	12.0	1,410	25.9	12.7	1,433	187:100
June 16	28.3	439	7,329	272	1.7	90	30	218	5.3	730	25.1	12.7	782	936:100
June 17	14.3	4	9,404	272	4.0	210	70	217	8.3	1,490	21.3	8.0	2,169	433:100
June 18	19.3	36	7,866	276	4.7	120	40	220	8.7	860	21.5	12.0	1,928	408:100
June 19	18.0	6	4,414	275	6.0	240	80	219	10.0	2,129	26.7	13.0	2,464	179:100
June 20	20.0		2,160									14.3	38	5,684:100
June 21	17.0		10,013									13.7	1,055	952:100
June 22	20.0		9,406									16.0	2,710	349:100
June 23	19.3		3,533									16.0	4,869	73:100
Daily average	20.7	68	5,037	273	3.9	176	59	231	9.6	1,408	28.2	12.9	1,817	295:100

TABLE 11

Summary of results of pregnant rats in experiment 4

DATE	CONTROL RATS					EXERCISED RATS								
						Forced work								
						Time in minutes								
	Grams food eaten in 24 hours	Voluntary run during period forced work	Total run for 24 hours	Weight at beginning forced work period	Weight lost during period	Total period	At work	Weight at beginning	Weight lost	Revolutions	Revolutions per minute	Grams food eaten in 24 hours	Total run for 24 hours	Ratio total run of control to exercised rats
June 1	22	21	10,652	251	1	180	60	259	11	1,127	18.8	16*	19,281	55:100
June 2	17	6	20,893	238	0	225	75	252	9	2,009	26.9	12	2,045	1,016:100
June 3	20	72	7,465	245	3	240	80	250	8	2,037	29.6	12	2,037	368:100
June 4	22	0	5,526	250	3	120	40	250	7	906	24.0	13	989	558:100
June 5	16	475	13,275	250	7	180	60	250	8	1,283	21.2	15	1,638	811:100
June 6	20*	0	27,821	242	4	120	40	252	14	925	23.1	15	1,562	1,781:100
June 7	18	31	3,374	248	4	150	50	256	8	1,022	20.4	16	1,504	224:100
June 8	21	152	7,418	254	5	180	60	260	10	1,036	17.8	17	1,931	384:100
June 9	23	107	6,620	255	1	180	60	262	9	1,316	21.9	17	2,350	294:100
June 10	24	1,000	4,116	260	4	240	80	266	14	2,246	28.1	19	3,270	126:100
June 11	24	399	1,973	263	5	135	45	266	14	1,179	26.2	20	1,321	149:100
June 12	26	398	3,985	270	4	195	65	268	11	1,800	27.7	17	2,117	188:100
June 13	21	60	1,759	268	4	135	45	270	14	1,229	27.3	18	1,874	94:100
June 14	28	332	4,473	282	8	240	80	271	12	2,227	27.7	18	3,318	135:100
June 15	24	164	2,854	280	0	165	55	274	10	1,425	25.9	18	3,092	97:100
June 16	34	1,019	1,832	292	3	90	30	276	10	752	25.1	24	821	223:100
June 17	20	50	3,674	290	5	210	70	275	9	1,495	21.3	9	6,869	53:100
June 18	27	7	3,282	298	0	120	40	282	6	862	21.5	21	1,173	279:100
June 19	22	3	3,559	302	7	240	80	295	17	2,133	26.7	22	4,700	76:100
June 20	24		2,225									20	1,541	146:100
June 21	22		1,582									22	2,254	70:100
June 22	30		2,140									21	2,926	73:100
June 23	22		1,308									24	2,216	59:100
Daily average	23	226	6,166	265	3.6	176	59	265	10.6	1,442	24.2	17.6	3,080	200:100

* Mated.

the pregnant rats the ratio between the control and the exercised animals was 131 to 100. These figures are somewhat greater than those of experiment 3 and seem to indicate a more economical use of the energy intake. The results, however, correspond to those of the preceding experiment in that the number of revolutions turned per gram of food eaten was greater in the control than in the exercised groups. This again indicates a greater expenditure of energy in a forced revolution than in a voluntary one. From the data in tables 10 and 11 we have computed the number of revolutions equivalent to the energy of one gram of food. We have found that in the control non-pregnant group it was 243 voluntary revolutions; in the control pregnant group it was 268 voluntary revolutions; in the exercised non-pregnant group it was 141 forced and voluntary revolutions; and in the exercised pregnant group it was 175 forced and voluntary revolutions. In each case the pregnant animals ran a greater number of revolutions per gram of food eaten than the corresponding groups of non-pregnant rats.

When the number of revolutions turned during the forced work period per gram loss in body weight are considered, we find that the number of revolutions turned by the exercised groups surpassed those of the control groups, both in non-pregnant and in pregnant animals. Again deducting the estimated loss due to resting metabolism the data show that per gram loss the non-pregnant controls ran 36 voluntary revolutions; the pregnant controls turned 141 voluntary revolutions; the non-pregnant exercised group ran 185 turns of forced work; and the pregnant exercised group did 168 forced revolutions. In this the exercised animals turned more revolutions per gram loss in weight than the controls in both non-pregnant and pregnant animals. With the exception of the non-pregnant control group a greater number of revolutions was turned per gram loss than in experiment 3.

The differences between the results in this experiment may have been partly due to the age of the individuals. At the beginning of experiment 3 the average age of the rats was 108 days. In this experiment the average age was 339 days at the beginning.

The daily weights of the animals at the beginning of the forced work also show some interesting results, which throw light on the food consumption. The weights are compared for the period from June 1 to June 19, during which time forced work was being given. The control non-pregnant rats had an average gain of eleven grams during this interval; the exercised non-pregnant animals lost 38 grams during the same time. This is significant. The 20.7 grams eaten by the control group was not only sufficient to furnish energy for the activity they performed, but supplied a surplus which was utilized in growth. The 12.9 grams of food taken by the exercised rats was insufficient and they were compelled to draw on their stored

Daily average.....	23	226	6,166	265	3.6	176	59	265	10.6	1,442	24.2	17.6	3,080	200:100

* Mated.

energy to accomplish the tasks imposed. The amount of food eaten, therefore, is not a true indicator of the energy turnover in the body, but must be considered in connection with the gain or loss in weight of the animal.

When the gains in body weight of the two groups of pregnant rats are considered for the same number of days after mating we find again that the control had a much larger gain than the exercised group. In the first thirteen days after mating the control female gained 54 grams while the exercised rat gained only 19 grams. The number of young born in each case would modify the results. The difference in the number of young of these pregnant females was not sufficient to account for the 35 grams discrepancy in gain in weight. The results therefore are in accord with those of the non-pregnant rats and can be explained in the same manner.

The results of this experiment substantiate those of preceding experiments. The minor differences may be due to individual variation. This experiment also shows that the intake of food is not a true measure of energy turnover, but that the gain and loss of body weight must be considered.

Experiment 5. This experiment was intended to demonstrate the effect of forced exercise during gestation on rats which had passed the prime of life. The animals used were the same as in experiment 4 and had a similar arrangement. At the beginning of forced work, July 26, 1924, they had the following weights, ages and dates of mating:

	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6	RAT 7	RAT 8
Weight	294	256	264	238	238	282	250	230
Age	407	407	407	402	402	402	402	402
Mated	7-20	7-29	7-24	7-24	7-20	7-20	7-21	7-25

This shows that coitus occurred in all the animals very near the date of beginning the forced work, the extremes being six days before and three days after July 26. The results of these matings were very unsatisfactory. Only two, 7 and 8, delivered young. Rat 3 failed completely, the curve of activity showing a typical four-day rhythm. The activity curves of rats 1, 2, 4, 5 and 6 resembled that of true gestation for periods varying from thirteen to twenty days. At this time typical oestrual peaks in activity occurred which proved that pseudopregnancy had resulted in these cases.

In this experiment all the forced exercise was given in the evening.

Since no comparison has been given in any of the preceding experiments relative to pseudopregnant rats, a brief summary will be given.

Table 12 gives the average results of control and exercised pseudopregnant rats. By consulting this table it is seen that the controls had a

greater total activity than the exercised rats, the ratio being 138 to 100 respectively. This corresponds to the results in the preceding experiments. It is also seen that during the time the exercised rats were forced to run 1581 revolutions the controls voluntarily ran only an average of 591 turns. This forms a ratio of 37 for the controls to 100 for the exercised rats. The amount of voluntary running during the remainder of the twenty-four hours was 3923 revolutions for the controls, and 1696 for the exercised rats, a ratio of 231 to 100 respectively. These results correspond favorably with those of preceding experiments in which the forced activity was given in the evening.

When the average daily food consumption is considered we find that the control rats averaged to consume 21.2 grams and the exercised rats 15.6 grams. This forms a ratio of 136 to 100 respectively. Comparing these figures with those of previous experiments we find them more closely related to the ratio between control and exercised pregnant rats than non-pregnant animals. If the number of revolutions per gram of food eaten are computed we find that the control rats averaged 213 revolutions while the exercised animals averaged 209 turns. These results are less than those of non-pregnant rats in experiment 4. This suggests a utilization of some of the energy of the food in other ways than in activity. As will be shown later, this was used in growth. As previously stated the amount of food eaten therefore is not a true measure of possible activity but must be considered also in relation to the gain or loss of body weight of the animal.

The average weight lost by the non-pregnant control rats during the average forced work period of 245 minutes was 3.96 grams. After deducting the estimated loss due to resting metabolism during this time we find that 1.96 grams loss were equivalent to 591 revolutions, or 301 turns per gram. This is greater than found in the preceding experiments. The total average weight lost by the non-pregnant exercised rats during forced work averaged 9.63 grams. Again deducting the loss due to resting metabolism we find that 7.63 grams loss were equal to 1581 revolutions of forced work, or 204 turns per gram loss. This shows again a greater efficiency than shown by younger rats. These results seem to indicate that older animals can perform a greater amount of work with a less expenditure of energy than young animals.

When we consider the pregnant rats in this experiment which were forced to run we find that they had the least total run of any (table 13). The ratio of the total average activity of non-pregnant control to pregnant exercised rats was 204 to 100 respectively. This compares very closely to the pregnant control and pregnant exercised rats of experiment 4. If the total average activity of the non-pregnant exercised rats is compared to that of the pregnant exercised animals the ratio is 151 to 100. This

TABLE 12

Summary of results of control and exercised pseudo-pregnant rats in experiment 5

DATE	CONTROL RATS					EXERCISED RATS								
						Forced work								
						Time in minutes								
	Average grams food eaten in 24 hours	Average voluntary run during period forced work	Average total run for 24 hours	Weight at beginning forced work period	Average weight lost during period	Total period	At work	Average weight at beginning	Average weight lost during forced work	Revolutions	Revolutions per minute	Average grams food eaten in 24 hours	Average total run for 24 hours	Ratio total run of control to exercised rats
July 26	25	320	5,058	263	0.7	60	20	260	8.5	474	23.7	23	1,955	259:100
July 27	24	138	3,737	267	2.0	95	32	264	10.0	567	17.7	19	979	381:100
July 28	24	198	7,630	267	1.3	120	40	261	9.0	801	20.0	20	1,293	650:100
July 29	28	87	7,369	271	0	180	60	264	13.0	1,187	19.1	18	1,668	448:100
July 30	25	1,018	4,272	274	1.3	150	50	259	8.0	1,133	22.6	20	1,887	226:100
July 31	24	544	1,973	280	2.7	225	73	260	10.5	1,599	21.9	16	2,972	66:100
August 1	25	1,774	5,130	261	6.7	270	90	265	11.0	1,377	15.3	13	1,781	288:100
August 2	19	199	985	287	5.7	300	100	249	9.5	1,873	18.7	11	2,757	36:100
August 3	24	1,732	3,500	287	3.3	240	80	248	10.0	1,416	17.7	10	4,778	73:100
August 4	17	157	637	287	1.0	240	80	247	7.0	1,405	17.6	14	3,870	16:100
August 5	22	158	2,138	291	4.0	300	100	248	9.5	1,956	19.5	11	3,386	63:100
August 6	25	1,264	2,371	293	7.0	360	120	246	9.0	2,502	20.8	14	4,234	56:100
August 7	21	724	1,773	294	8.0	360	120	246	12.5	2,699	22.5	14	4,651	38:100
August 8	18		3,443									12	4,114	84:100
August 9	17	226	8,175	288	6.0	300	100	245	11.0	2,126	21.3	16	6,038	135:100
August 10	17	858	5,492	285	6.7	360	120	245	12.0	2,600	21.6	15	3,167	173:100
August 11	15	59	8,397	283	6.7	360		244	5.0	0		14	1,300	645:100
August 12	15		4,224									17	6,685	63:100
August 13	15		9,458									20	4,759	198:100
Daily average	21.2	591	4,514	280	3.96	245	82	253	9.63	1,581	20.0	15.6	3,277	138:100

indicates that the non-pregnant rats were not so easily fatigued as the pregnant animals.

In regard to the food consumption of the exercised pregnant animals we find that it was more than the non-pregnant exercised animals, but less than the non-pregnant controls. This difference would be expected, due to the developing young. In the pregnant exercised rats one gram of food was equivalent to 122 revolutions which is considerably less than either of

TABLE 13

Summary of results of exercised pregnant rats in experiment 5

DATE	FORCED WORK						AVERAGE GRAMS FOOD EATEN IN 24 HOURS	AVERAGE TOTAL RUN FOR 24 HOURS	RATIO TOTAL RUN OF CONTROL PSEUDOPREGNANT TO EXERCISED PREGNANT RATS	RATIO TOTAL RUN OF EXERCISED PSEUDOPREGNANT TO EXERCISED PREGNANT RATS
	Time in minutes		Average weight at beginning	Average weight lost during forced work	Average revolutions	Revolutions per minute				
	Total period	At work								
July 26	60	20	240	8.0	512	25.6	22	2,008	250:100	98:100
July 27	95	32	241	6.5	566	17.7	18	980	380:100	100:100
July 28	120	40	246	11.0	796	19.9	22	2,040	374:100	63:100
July 29	180	60	249	11.0	1,178	19.2	19	2,860	257:100	58:100
July 30	150	50	249	10.0	1,126	22.5	20	1,896	224:100	100:100
July 31	225	73	256	15.0	1,591	21.8	19	2,223	89:100	134:100
August 1	270	90	253	14.0	1,722	19.1	17	1,798	285:100	99:100
August 2	300	100	253	12.5	1,870	18.7	17	2,078	475:100	132:100
August 3	240	80	255	10.0	1,402	17.8	19	1,658	211:100	283:100
August 4	240	80	259	13.0	1,400	17.5	16	2,660	24:100	145:100
August 5	300	100	261	13.0	1,945	19.4	16	2,338	91:100	145:100
August 6	360	120	263	16.0	2,454	20.4	18	3,564	67:100	119:100
August 7	360	120	262	16.0	2,685	22.4	16	3,495	51:100	133:100
August 8							16	42		979:100
August 9	300	100	271	16.0	2,110	21.1	20	4,678	175:100	129:100
August 10	360	180	270	15.0	2,587	14.8	16	2,817	195:100	113:100
August 11	360	180	273	18.0	2,052	11.4	15	2,360	355:100	55:100
August 12							14	288	1,469:100	2,321:100
August 13							19	1,463	647:100	326:100
Daily average	245	82	256	12.8	1,623	19.3	17.8	2,171	204:100	151:100

the other two groups in this experiment, and less than the exercised pregnant rats in experiment 4 but greater than in experiment 3. This again suggests a better utilization of the food energy by older rats than by younger ones.

When the average daily weights of the animals during the days of forced exercise are considered we find that the control non-pregnant rats gained 20 grams in the 17 days. This shows that some of the food energy

was used in increasing the body weight. In the case of the non-pregnant exercised rats whose average loss in weight during the seventeen days of forced work was 16 grams, the average daily intake of 15.6 grams was inadequate to meet the demands and stored energy had to be utilized. The body weight of the pregnant exercised rats, due to the developing young, increased 33 grams. At the same time they had a daily average food intake of 17.8 grams which was greater than that of the non-pregnant exercised rats. In order to determine if this amount of food was sufficient to meet the requirements of the forced work and the increased weight, a comparison is made between the results of these animals and the pregnant control rats of experiment 4. This shows that the gain in weight during the last seventeen days of forced work of the controls of experiment 4 was 57 grams while the exercised pregnant rats in the same experiment gained only 45 grams. This lower gain in weight indicates that in both these experiments the exercised pregnant rats did not consume enough food to equal the demands of growth of young and the forced activity, but, like the other exercised rats, had to use some of their stored energy. This resulted in a less gain in weight than that exhibited by the control animals.

While the results of this experiment in general accord with those of the preceding experiments, they seem to strongly indicate that pseudopregnant rats are affected by forced exercise less than pregnant animals, but more than non-pregnant ones. The results also suggest that older rats are not so greatly affected by forced work as younger animals.

Experiment 6. The animals used in this experiment were relatively young at the beginning of forced exercise. The controls averaged 182 days of age, the youngest being 179 and the oldest 183 days. The exercised group averaged 180 days, the youngest being 179 and the oldest 182 days. The average weight of the controls was 204 grams and of the exercised group 200 grams. Matings were made between September 14 and September 19. These animals were in their prime. They had almost the same age and weight, and were mated at as nearly the same age as possible. They were placed in the revolving cages August 26 and soon showed typical oestrual rhythm in their voluntary activity. The forced work, which in all cases was given in the evening, was begun September 20 and was given for 18 consecutive days. A summary of the average results is given in table 14.

With the exception of rat 4, which continued to show typical oestrual rhythm, all the controls showed that copulation had taken place. Pseudopregnancy resulted in rats 1 and 3, typical oestrual cycles appearing on the thirteenth and fourteenth days after mating. They were again remated and rat 3 delivered young. Seven days after second mating rat 1 again showed typical oestrual cycles which continued in a four day rhythm throughout the experiment. There were no indications of pseudopregnancy or oestrual cycles in the exercised rats. They all appeared to be pregnant.

Exercised rat 6 died October 1 during the twelfth period of forced work at the age of 191 days and on the sixteenth day of gestation. At the beginning of this period of forced work she weighed 196 grams and at death 181 grams. This rat weighed 196 grams at the beginning of the forced work periods, September 20, which was the fifth day of gestation. There was a gradual loss of weight to 190 grams on the ninth day followed by an increase to 204 grams on the thirteenth day of gestation. Notwithstanding the constant increase in weight of the developing young, which in most other cases of exercised pregnant rats caused an increase in body weight of the mother, this rat again lost weight reaching 196 grams on the day of her death. The autopsy showed the uteri badly congested. The left uterus showed signs of nine embryos. Six were apparently normal and weighed, freed from placenta and amniotic fluid, 2.4 grams. Resorption had taken place in the other three to the extent that only a trace was visible of one, another was almost wholly gone, while the third was about one-half resorbed. The right uterus had five embryos, one almost wholly resorbed and the other four, which weighed 1.65 grams, were apparently normal. The average weight of all the normal embryos was 0.405 gram. This is 0.095 gram heavier than that given by Stotsenburg and Donaldson for sixteen day old fetuses, and corresponds with the results in our preceding experiment, no. 3. The left ovary had eleven corpora lutea and the right had six. The total weight of the embryos and placenta was 7.5 grams. All other organs were normal. The conclusions were that this rat died from overwork.

After the death of exercised rat 6, control rat 2 was transferred from its cage to cage 6 and given forced work. This rat was chosen because its voluntary activity curve had showed no oestrual cycles and its increase in weight suggested that it was pregnant. Its average voluntary run for the preceding ten days was 5963 revolutions. The first day the forced exercise period lasted five and one-half hours during which time it was compelled to run 2117 revolutions. During this time it lost 19 grams in weight while the other exercised rats, which had become accustomed to the forced work, lost only 10 grams. For the remainder of the twenty-four hours this rat ran voluntarily only 52 revolutions. On the second evening of forced exercise after two and one-half hours and 1280 forced revolutions it died. During this time it lost 17 grams while the other exercised rats lost 8 grams. The weight of this rat on the day it was transferred to cage 6 was 225 grams. Its weight at death was 193 grams. It thus lost 32 grams in the two days. Unlike the other exercised rats which were accustomed to the forced work it was able to recuperate only a small part of the daily loss. Autopsy showed all organs normal except the left ovary which was somewhat congested. If this animal had been pregnant, resorption had been complete. Since coitus occurred in

this rat fourteen days previous to its death this may have been a case of pseudopregnancy. This experiment indicates that the rat cannot endure the strain of forced work if subjected at once to long periods without previous and gradual training. These results and those of experiment 1 show that this is true of both non-pregnant and pregnant rats.

By consulting table 14 we see that the ratio of total daily activity of control to exercised rats was 325 to 100. This is greater than in any of the preceding experiments. This was probably due to the fact that some of the control rats were non-pregnant, some pseudopregnant part of the time and some pregnant. The ratio of the voluntary run of the controls during the period of forced work to the forced exercise of the exercised rats was 45 to 100. This is also greater than in any of the preceding experiments. The ratio of the food consumption of the control to the exercised rats was 144 to 100. This ratio was only exceeded by the non-pregnant rats of experiment 4. The next nearest to this was the pseudopregnant animals of experiment 5. The number of revolutions per gram of food eaten was 304 for the control and 134 for the exercised rats. The controls, in this case, turned a greater number of revolutions per gram of food eaten than in any of the preceding experiments. The number for the exercised rats was about an average. Calculating, as in previous experiments, the number of revolutions per gram of body weight lost we find that in the controls one gram loss was equal to 210 revolutions and in the exercised group it was 199 revolutions. These figures are not far from the average. During the eighteen days of forced work the controls gained 32 grams while the exercised rats lost 16 grams. A gain of 32 grams is about normal for non-pregnant females of this age. The loss of 16 grams in the exercised rats was exactly the same as that lost by the pseudopregnant exercised rats in experiment 5. The rats in this experiment, however, were forced to run daily over 200 revolutions more than those of experiment 5 and to run one day longer. The non-pregnant exercised rats in experiment 4 lost 38 grams. From these data one can assume that the exercised rats in this experiment were either pregnant or pseudopregnant. None of them delivered young. We know from the autopsy of no. 6 that she was pregnant and that the young were being resorbed. The daily weights and activity curves of the other exercised rats corresponded so closely to those of no. 6 up to her death that it indicates pregnancy and complete resorption in them. No other evidence is available at this time.

The results of this experiment substantiate, in general, those of the preceding experiments. They also indicate that from the thirteenth to the sixteenth or seventeenth day after coitus is a critical time in the life of the female rat. This experiment also shows that not only may resorption of embryos be caused by excessive compulsory work but also that the death of the mother may result. It also indicates that the rat cannot

TABLE 14

Giving average results of control and exercised rats in experiment 6

DATE	CONTROL RATS				EXERCISED RATS								
					Forced work								
					Time in minutes								
	Average grams food eaten in 24 hours	Average voluntary run during period forced work	Average total run for 24 hours	Average weight at beginning forced work period	Average weight lost during work period	Total period	At work	Average weight at beginning	Average weight lost during forced work	Revolutions	Revolutions per minute	Average grams food eaten in 24 hours	Average total run for 24 hours	Ratio total run of control to exercised rats
September 20	17	0	4,739	204	5.6	120	40	200	14.7	529	13.2	13	2,610	181:100
September 21	19	626	5,383	205	2.5	180	60	198	11.5	1,012	16.9	15	1,045	538:100
September 22	18	361	5,269	206	2.5	270	90	195	12.8	1,799	18.6	16	2,053	341:100
September 23	20	145	5,606	209	5.0	270	90	190	11.8	1,851	20.5	13	1,916	292:100
September 24	21	720	7,590	211	4.8	270	90	190	10.8	1,852	20.5	15	2,402	315:100
September 25	22	650	6,888	212	5.7	300	100	191	10.5	2,134	21.3	17	2,170	317:100
September 26	22	34	7,860	216	4.0	180	60	192	8.0	1,307	21.3	16	1,386	567:100
September 27	21	1,484	8,130	220	7.2	330	110	195	11.5	1,672	15.2	17	1,750	464:100
September 28	22	1,536	6,542	220	6.0	300	100	200	14.0	2,542	25.4	14	2,560	255:100
September 29	23	1,229	6,814	225	8.2	255	64	193	15.5	2,006	31.3	14	2,043	333:100
September 30	19	1,444	7,006	222	5.8	300	100	188	19.0	2,308	23.1	12	2,326	301:000
October 1	20	1,750	9,458	223	5.0	335	112	189	12.3	2,814	25.1	12	2,828	335:100
October 2	20	856	5,863	223	4.7	315	104	187	10.0	2,117	20.3	14	2,158	272:100
October 3	25	391	5,775	225	3.7	210	70	190	8.0	1,280	14.0	17	1,409	410:100
October 4	25	733	6,179	226	4.0	210	70	192	8.7	1,412	20.2	19	1,724	356:100
October 5	20	507	5,698	230	3.0	210	70	190	8.3	1,642	23.3	15	2,208	330:100
October 6	20	806	6,690	234	6.7	300	100	188	9.0	2,147	21.5	10	2,207	313:100
October 7	29	1,055	5,100	236	5.3	270	90	184	6.7	1,909	21.2	18	2,007	255:100
Daily average	21.3	801	6,474	219	6.0	256	85	191	11.3	1,796	21.0	14.8	1,988	325:100

endure excessive forced work without previous and gradual training. Daily weights and activity curves of the exercised rats strongly indicate that they were all pregnant and that complete resorption occurred in those which lived throughout the experiment.

Experiment 7. This experiment was conducted differently from any of the preceding experiments. A belt was extended over all the cages so that forced work could be given to all the rats. Since there had always been matings which had later proved to be unsuccessful, we hoped this would give us a larger per cent of fertile matings which had received forced work from an early date in gestation. This arrangement also permitted a comparison of the effect of forced work on pregnant and non-pregnant rats under the same conditions relative to climatic changes, time of year, etc., without shifting them from their own cages. It also enabled us to determine the effect of compulsory work on a rat which had delivered and eaten her young a day previous to a series of periods of forced exercises and to make comparisons of these results with those of non-pregnant and pregnant animals.

The rats used in this experiment were the same as in experiment 6 with the exception of former rats 2 and 6 which had died. These were replaced by new rats. All the animals were approximately the same age, ranging between 232 and 238 days at the beginning of forced work. The average weight was 249 grams. Ample time had elapsed since the termination of experiment 6 for complete recovery and for the new rats to become accustomed to turning their cages.

Mating attempts were made with all the rats between October 22 and November 8. Rats 2, 4 and 8 soon showed by their activity and weight curves that they were not pregnant. All the others appeared to be pregnant. Rat 5, however, later proved to be pseudopregnant and began regular oestrual cycles twelve days after copulation. We thus had four rats, 2, 4, 5 and 8, which were not pregnant and four, 1, 3, 6 and 7, which were pregnant for comparison. Both groups were exercised practically the same each period. Owing to the fact that pseudopregnant rats behave more like pregnant than non-pregnant animals, the records of rat 5 were not used in computing the average results. Rat 7, which was mated early, gave birth to eight young on the third day of forced work. The gestation period was less than twenty-two days, the exact time not being ascertained owing to parturition occurring in the night. This rat was no longer given forced exercise nor its records used in the average results. Rat 1 gave birth to two young, which she immediately ate, after the ninth period of forced work. The gestation period was twenty-one days and nineteen hours. There was but one day's stop in the giving of forced exercise in this case. The records of this rat after delivery were so different from that of any of the others that they were arranged in a group by themselves.

The forced exercise was commenced November 11 and continued daily for twenty-one days. In all cases it was given in the evening. As in all other experiments the forced work began with a small run and gradually increased to the maximum. This probably accounts for the safe delivery of young by rat 7 and not her death as resulted in experiment 1. Another fact which was probably also an influencing factor was that this rat had become accustomed to the forced work in experiment 6. This latter fact may also account for the delivery of young by rat 1 on the ninth day of forced work and the forced work the cause of her eating them.

With the exceptions of rats 1 and 6 which were mated early none of the other rats produced young. The forced exercise apparently resulted in pseudopregnancy in rat 5 and in complete resorption of the young in the other rats whose records indicated pregnancy.

The average results of the non-pregnant and pregnant rats are given in table 15. By consulting this table it is seen that the average total daily activity of the non-pregnant rats was greater than that of the pregnant animals, the ratio being 117 to 100 respectively. This ratio is considerably less than gotten in former experiments in which non-exercised and exercised groups were compared. Since all these rats were given forced exercise this indicates, as previously stated, that a revolution of forced work required a greater expenditure of energy than a revolution voluntarily turned. It also shows that the non-pregnant rats were less affected by the compulsory work than the pregnant animals.

When the average daily food consumption is considered we find that there was only a slight difference. The non-pregnant animals averaged 16.5 grams daily while the pregnant rats averaged 16.1. If the number of revolutions per gram food eaten is considered, the non-pregnant rats averaged 224 revolutions and the pregnant animals 187 revolutions. That this difference in food intake was insufficient to account for the much greater activity of the non-pregnant rats is indicated by their greater loss in weight during the forced work periods. That is, they also used a greater amount of their stored energy. The non-pregnant rats lost 15 grams while the pregnant ones lost only 7 grams during the twenty-one days of forced work.

During the average daily work period of 310 minutes the average loss in body weight of the non-pregnant rats was 12.7 grams and of the pregnant animals 12.9 grams. After deducting the loss due to resting metabolism we find that per gram body weight lost during the forced exercise the non-pregnant rats averaged 230 and the pregnant ones averaged 223 revolutions. This slight difference can probably be accounted for by the greater average body weight of the pregnant rats which would require an increased expenditure of energy per revolution. These results show a more economic expenditure of energy in these rats than in any exercised

TABLE 15

Giving average results of non-pregnant exercised and pregnant exercised rats in experiment 7

DATE	NON-PREGNANT						TOTAL PERIOD OF FORCED WORK	MINUTES AT WORK	PREGNANT						RATIO TOTAL RUN OF NON-PREGNANT TO PREGNANT RATS	RATIO OF FOOD NON-PREGNANT TO PREGNANT	RATIO REVOLUTIONS PER GRAM LOSS NON-PREGNANT TO PREGNANT
	Average grams food eaten in 24 hours	Average revolutions of forced work	Average revolutions per minute	Average total run for 24 hours	Average weight at beginning forced work	Average weight lost during forced work			Average revolutions of forced work	Average revolutions per minute	Average total run for 24 hours	Average weight at beginning forced work	Average weight lost during forced work	Average grams food eaten in 24 hours			
November 11	15.0	1,057	17.6	3,800	235	12.7	180	60	1,057	17.6	1,471	259	10.5	14.0	258:100	107:100	121:100
November 12	15.7	1,545	19.3	1,891	230	12.7	240	80	1,523	19.1	1,740	259	12.5	18.5	109:100	125:100	102:100
November 13	14.3	1,734	18.3	2,573	228	12.0	285	95	1,711	18.0	2,141	258	12.7	13.5	120:100	106:100	94:100
November 14	13.7	2,038	18.5	3,227	224	12.0	330	110	2,010	18.3	2,527	247*	14.3	15.7	128:100	87:100	84:100
November 15	14.7	2,157	17.9	3,209	222	14.3	360	120	2,133	17.7	2,392	246	14.3	17.3	134:100	85:100	100:100
November 16	17.0	1,720	17.2	2,807	221	12.7	300	100	1,709	17.1	3,363	247	11.3	19.3	83:100	88:100	112:100
November 17	14.3	2,367	19.7	2,396	219	11.7	360	120	2,333	19.7	4,965	237	13.3	15.7	48:100	91:100	88:100
November 18	18.7	1,765	19.6	1,944	219	12.3	270	90	1,750	19.5	4,354	246	9.7	18.3	45:100	102:100	127:100
November 19	16.3	2,126	21.3	2,327	222	15.3	300	100	2,102	21.0	2,192	248	14.3	18.0	106:100	91:100	107:100
November 20	17.0	3,161	26.2	3,384	219	11.7	360	120	3,146	26.0	4,028	251	15.0	15.3	84:100	111:100	78:100
November 21	17.0	3,458	26.5	5,303	225	14.3	390	130	3,433	26.4	4,491	236†	12.5	14.0	118:100	121:100	102:100
November 22	18.0	2,480	22.5	3,327	221	12.3	330	110	2,456	22.3	2,626	232	13.5	13.0	128:100	138:100	95:100
November 23	19.7	1,783	22.8	1,936	223	12.0	235	78	1,723	22.6	1,792	227	12.5	16.0	108:100	123:100	96:100
November 24	17.3	2,045	18.6	5,477	221	14.7	330	110	2,016	18.3	2,702	229	14.0	13.5	202:100	128:100	105:100
November 25	19.0	1,731	21.6	5,040	228	12.7	240	80	1,708	21.4	1,855	230	11.5	16.0	269:100	119:100	110:100
November 26	18.0	2,967	27.0	4,908	231	13.0	330	110	2,928	26.6	3,075	231	13.5	13.5	160:100	133:100	96:100
November 27	15.0	2,492	24.4	3,737	226	12.3	300	100	2,457	24.6	3,454	226	11.0	18.0	108:100	136:100	112:100
November 28	15.7	3,339	27.8	3,924	228	13.7	360	120	3,286	27.4	3,477	228	11.5	14.5	113:100	136:100	119:100
November 29	13.3	3,145	28.6	3,687	225	14.3	330	110	3,105	28.2	3,384	227	20.5	17.0	109:100	78:100	70:100
November 30	18.0	3,000	25.0	4,913	221	11.0	360	120	2,958	24.6	3,401	229	10.0	18.5	144:100	180:100	180:100
December 1	19.0	2,829	25.7	4,507	220	9.3	330	110	2,785	25.4	3,782	229	12.0	18.0	119:100	105:100	77:100
Daily average	16.5	2,330	22.2	3,533	224	12.7	310	103	2,301	22.0	3,010	238	12.9	16.1	117:100	104:100	99:100

* Records of rat 7 stopped on account of birth of young.

† Records of rat 1 stopped on account of birth of young.

rats in the former experiments. This may have been due to the former training gotten in experiment 6.

As previously mentioned, the records of rat 1 will be considered separately. This rat presented a condition quite different from any others so far considered. She was successfully mated at 9 p.m. October 29 and after nine days of forced work delivered two young at 4 p.m., November 20. The gestation period was twenty-one days and nineteen hours. The young were eaten within one day. This rat weighed 278 grams at the beginning of forced exercise, November 11, and on the day prior to delivery 279 grams. During these nine days the average daily loss in weight during the forced work was 16 grams and the average daily food intake was 15.6 grams. One day after the young were eaten this rat was again given forced exercise for seven consecutive days. On the seventh day, after five and

TABLE 16

Comparison non-pregnant, pregnant and new mother (just after eating her young) during seven days of forced work (November 11 to 28 inclusive). Experiment 7

	Average daily forced work	Average daily voluntary run	Average total daily run	Body weight		Average daily weight lost	Average daily food eaten	Revolutions per gram weight lost during forced work	Revolutions per gram food eaten
				Gain	Loss				
Non-pregnant	2,405	1,645	4,050	7		13.3	17.2	224	235
Pregnant	2,382	329	2,711		4	12.5	14.8	238	183
New mother	2,358	16	2,374		27	18.0	11.7	152	202

one-half hours of forced work, the rat was found completely exhausted and rolling in the cage. Death would very probably have resulted if the forced work had continued to the end of the period. After this time forced exercise was no longer given owing to the very feeble condition to which this rat had been reduced. For several days following, this animal was in a very feeble condition and did very little voluntary running. Eleven days elapsed before she regained her normal weight and voluntary activity.

Table 16 gives in condensed form the results of rat 1 with a comparison of the findings of the non-pregnant and pregnant rats for the same days. The range from 1645 revolutions in the non-pregnant to 16 in the new mother in the amount of average daily voluntary run after forced exercise is very conspicuous. This is a good indication of the effect of the work on these three groups of animals. The effect on body weight is also very

* Records of rat 7 stopped on account of birth of young.
† Records of rat 1 stopped on account of birth of young.

noticeable. During these seven days the non-pregnant rats gained 7 grams, the pregnant animals lost 4 grams, while the new mother lost 27 grams. The average daily loss in weight during forced work is also conspicuous. The non-pregnant rats averaged to lose 13.3 grams daily, all of which, and a little more, they were able to regain during their resting period. The pregnant rats averaged to lose 12.5 grams and were almost successful in regaining this loss. But the new mother averaged to lose 18 grams daily only a portion of which she was able to regain. This was partly due to a lower consumption of food and partly to the enfeebled condition which resulted.

The results of this experiment substantiate in many ways those previously described. They further seem to indicate that forced exercise affects new mothers most, pregnant rats less, and non-pregnant rats least of all. They also suggest that previous training is of great benefit to the individual. The fact that only the rats which were mated early and were subjected to forced work for a comparatively short time, delivered litters, strongly suggests that continued forced work prevents, or inhibits, the normal reproductive processes by lack of insemination, fertilization, or implantation, or by resorption or possible abortion.

Experiment 8. In this experiment we endeavored to have all of the eight rats used not only pregnant, but mated at as nearly the same time as possible. This was accomplished by having eighteen breeding females in revolving cages and selecting those whose peaks of activity occurred on approximately the same day. We were successful in securing copulation within twenty-four hours by each of the eight rats selected for use. The exact hour of mating was noted in order to compute the gestation period. Vaginal smears showed that insemination had resulted in each individual. We thus had animals which had been accustomed to turning the revolving cages for more than a month that were bred at approximately the same time. Three of the rats, 1, 3 and 7, had been previously used in experiments 6 and 7 and were accustomed to forced work. The remaining ones had never been subjected to forced exercise, but their average voluntary run had been more than double the revolutions they were later forced to run. The average age was 224 days and the range was from 162 to 280 days. The rats from experiment 7 were 280 days of age. The others consisted of two groups of sisters 162 and 232 days of age respectively at the beginning of the forced work. The average weight of the eight rats was 220 grams and the range was from 188 to 272 grams. The oldest rats were the heaviest and had practically reached their maximum weight while the others were still growing. We thus had three groups of individuals consisting of fully matured, almost full grown, and growing rats. Rats 1, 3 and 7 had previously copulated and 3 and 7 had delivered young. All the other rats were virgins.

The forced work was begun December 28 and continued daily for nineteen days. In all cases the exercise was given in the afternoon and evening between the hours of 2 and 10 p.m. As in the other experiments the first day's work was relatively short, being of only three hours duration. The longest period was eight hours. In no case was the number of forced revolutions equal to the average voluntary run the rats had previously exhibited. The total exercise periods amounted to approximately 23.5 per cent of the nineteen days.

The weights of the animals were taken just before and after each work period. The loss in body weight was ascertained and the energy equivalent per gram loss, as indicated by revolutions of forced work, was determined. Individual curves of weight, activity and food intake were plotted daily. A summary of the average results is given in tables 17, 18 and 19 and graphically shown in figures 1, 2 and 3.

The forced exercise was discontinued January 15, two days prior to the normal time of delivery. All the young were born on January 17 between the hours of 9:40 a.m. and 5:15 p.m. The gestation periods ranged between 21 days, 13 hours to 22 days, 15 minutes. The older rats had the longer gestation periods. The mother which delivered the fewest young had the longest gestation period. This accords with results formerly obtained (6). The average gestation period for the group was 21 days, 18.5 hours. This was approximately eight hours longer than the general average of rats kept in revolving cages under normal conditions of exercise as previously published (6). Since the average size of the litters was only slightly below the normal we are led to conclude that the forced exercise given tended to lengthen the gestation period.

Only four of the eight rats, 1, 3, 5 and 7, delivered young. The others, 2, 4, 6 and 8, were pseudopregnant as indicated by their weight and activity curves. That pseudopregnancy in rat 2 was due to resorption is shown by the autopsy findings given later. We have no means of determining definitely whether pseudopregnancy in rats 4 and 6 was due to the same cause. The activity and weight records, however, strongly suggest that resorption occurred. There is very strong evidence that rat 8 aborted January 10, the twelfth day of forced work and the fifteenth day after coitus. On this date the vaginal opening and nest were bloody. If young had been delivered they had been eaten. We feel justified in stating that abortion occurred in this rat. We thus had only 50 per cent of the normal healthy rats which had been inseminated that delivered young. This is much below the normal and leads us to conclude that compulsory forced work during gestation is harmful to normal reproduction.

Rat 2 was found dead at 7 p.m., January 11, after four and one-half to five hours of forced work. During this time she ran 2447 revolutions and lost 14 grams in weight. This was the fifteenth day of forced work and

TABLE 17

Giving the average daily results of the pseudopregnant and pregnant rats during the nineteen days of forced exercise in experiment 8

DATE	PSEUDOPREGNANT								TOTAL PERIOD FORCED WORK IN MINUTES	MINUTES AT WORK	PREGNANT							
	Average grams food eaten in 24 hours	Average revolutions of forced work	Average revolutions per minute	Average total run for 24 hours	Average weight at beginning forced work	Average grams lost during forced work	Per cent loss	Revolutions per gram loss			Average grams food eaten in 24 hours	Average revolutions of forced work	Average revolutions per minute	Average total run for 24 hours	Average weight beginning forced work	Average grams lost during forced work	Per cent loss	Revolutions per gram lost
December 28	17	1,026	17	5,396	207	9	4.3	114	180	60	20	1,025	17	2,911	253	11	4.3	92
December 29	16	1,333	17	1,721	206	10	4.8	133	240	80	19	1,332	17	2,264	254	14	5.5	95
December 30	17	1,523	16	1,586	206	12	5.8	127	285	95	22	1,522	16	2,318	257	15	5.8	101
December 31	16	1,974	25	2,088	207	13	6.2	152	240	80	21	1,985	25	2,484	259	18	6.9	110
January 1	15	2,315	26	2,484	204	12	5.8	192	270	90	19	2,314	26	2,826	253	14	5.6	165
January 2	14	1,982	25	2,135	204	11	5.4	180	240	80	20	1,980	25	2,211	252	15	6.0	132
January 3	20	2,611	26	2,886	205	12	5.8	217	300	100	22	2,611	26	3,668	252	12	4.8	217
January 4	17	2,786	21	2,976	205	11	5.3	253	390	130	23	2,787	21	3,406	258	14	5.4	199
January 5	15	2,656	20	2,919	208	10	4.8	265	390	130	19	2,652	20	3,122	259	12	4.6	221
January 6	17	2,846	22	3,224	210	12	5.7	237	390	130	18	2,854	22	3,353	259	15	5.8	190
January 7	17	3,140	21	3,291	208	16	7.7	196	445	148	22	3,137	21	3,450	260	12	4.6	261
January 8	17	2,442	19	3,275	200	12	6.0	203	390	130	21	2,434	19	3,319	258	11	4.2	221
January 9	17	3,211	19	3,470	204	15	7.3	208	480	160	21	3,125	19	4,499	262	16	6.1	195
January 10	17	2,755	23	3,303	205	15	7.3	183	360	120	25	2,756	23	3,492	265	17	6.4	162
January 11	16	2,824	23	6,650	196*	11	5.6	257	360	120	26	2,931	24	3,527	266	14	5.2	209
January 12	16	3,063	23	3,446	208	13	6.2	236	390	130	20	3,070	24	3,337	272	15	5.5	205
January 13	20	2,912	24	3,640	209	11	5.2	264	360	120	26	2,916	24	3,325	276	14	5.1	208
January 14	15	3,075	23	3,457	206	13	6.3	236	390	130	23	3,083	24	3,570	287	12	4.2	257
January 15	19	2,291	21	5,925	204	9	4.4	254	315	105	21	2,296	22	2,466	288	16	5.5	143
Average	16.7	2,454	21.6	3,411	205	11.9	5.78	205	338	113	21.5	2,463	21.8	3,135	262	14	6.05	178

* Rat 2 died.

fifteen days and twenty hours after coitus and insemination had occurred. It seems more than coincidence that several of our rats have died about fifteen days after successful copulation. At the beginning of the experiment this rat weighed 218 grams. At her death she weighed 165 grams, having lost 53 grams during the fifteen intervening days. The curve of daily weight shows an almost uniform drop. This rat was in the prime of life, being only 248 days of age at death. The daily food consumption

TABLE 18

Continued from table 17. Giving data relative to food consumption, activity and weight during recovery from forced exercise in experiment 8

DATE	PSEUDOPREGNANT			PREGNANT		
	Average grams food eaten in 24 hours	Average total revolutions in 24 hours	Average weight	Average grams food eaten in 24 hours	Average total revolutions in 24 hours	Average weight
January 16	22	786	203	20	179	290
January 17	21	6,025	203	19	759	250*
January 18	24	5,334	204	19	873	249
January 19	19	10,826	211	17	374	255
January 20	28	9,090	215	26	675	257
January 21	25	9,960	216	29	1,270	260
January 22	26	8,185	214	31	634	262
January 23	21	10,862	215	22	876	259
January 24	18	11,718	219	29	2,085	260
January 25	25	13,064	223	33	702	277
January 26	25	9,789	234	34	805	290
January 27	24	13,545	229	33	901	285
January 28	19	15,538	227	38	900	291
January 29	25	18,581	225	42	1,148	291
January 30	23	16,408	228	37	1,288	294
Daily average	23	10,647	248	29	897	271
Weight gained			25			44

* Young born.

during the fifteen days was about the average and on only three days did the number of grams eaten fall slightly below that of any of the other animals. The amount of forced work given this rat was apparently about the limit of her endurance, for during the resting periods she voluntarily ran the least number of revolutions of any of the group. This was especially noticeable during her last eight days during which she averaged to run voluntarily only a little over four revolutions daily. This may

TABLE 19

Giving the ratios of average total daily activity, food consumption, revolutions per gram loss, body weights, and per cent loss of pseudopregnant to pregnant rats in experiment 8

DATE	RATIO OF TOTAL RUN OF PSEUDOPREGNANT TO PREGNANT RATS	RATIO OF FOOD EATEN—PSEUDOPREGNANT TO PREGNANT RATS	RATIO REVOLUTIONS PER GRAM LOSS—PSEUDOPREGNANT TO PREGNANT RATS	RATIO BODY WEIGHT—PSEUDOPREGNANT TO PREGNANT RATS	RATIO PER CENT LOSS—PSEUDOPREGNANT TO PREGNANT RATS
December 28	185:100	85:100	124:100	82:100	100:100
December 29	76:100	84:100	140:100	81:100	87:100
December 30	69:100	77:100	126:100	80:100	100:100
December 31	84:100	76:100	138:100	80:100	90:100
January 1	88:100	79:100	116:100	81:100	104:100
January 2	96:100	70:100	136:100	81:100	90:100
January 3	79:100	91:100	100:100	81:100	121:100
January 4	87:100	94:100	127:100	80:100	98:100
January 5	93:100	79:100	120:100	80:100	104:100
January 6	96:100	94:100	125:100	81:100	98:100
January 7	95:100	77:100	75:100	80:100	167:100
January 8	99:100	81:100	92:100	78:100	143:100
January 9	77:100	81:100	107:100	78:100	120:100
January 10	95:100	68:100	113:100	77:100	114:100
January 11	189:100	62:100	123:100	74:100	108:100
January 12	103:100	80:100	115:100	76:100	113:100
January 13	109:100	77:100	127:100	76:100	102:100
January 14	97:100	65:100	88:100	72:100	150:100
January 15	240:100	90:100	157:100	71:100	80:100
January 16	439:100	110:100		70:100	
January 17*	743:100	110:100		81:100	
January 18	610:100	126:100		83:100	
January 19	2,891:100	112:100		84:100	
January 20	1,347:100	108:100		83:100	
January 21	784:100	86:100		82:100	
January 22	1,291:100	84:100		83:100	
January 23	1,240:100	96:100		84:100	
January 24	563:100	62:100		81:100	
January 25	1,860:100	76:100		81:100	
January 26	1,214:100	73:100		81:100	
January 27	1,503:100	73:100		80:100	
January 28	1,724:100	50:100		78:100	
January 29	1,626:100	60:100		77:100	
January 30	1,404:100	62:100		77:100	
Average first 19 days	105:100	78:100	118:100	78:100	106:100
Average last 15 days	1,286:100	86:100		80:100	
Total average	627:100	82:100	118:100	79:100	106:100

* Young born.

have been due to the condition of her feet. Due to her persistent sliding in the cage it was very hard to keep her feet in good condition and free from abrasions.

The autopsy of this animal showed the following organs normal: heart, lungs, stomach (partly filled), intestines, pancreas, liver, spleen, kidneys, adrenals, ovaries and left horn of the uterus. The right horn of the uterus showed evidence of resorption which had almost cleared up. A darkened portion about 15 mm. in length was all that remained. Seven corpora lutea of different sizes were counted on the right ovary and sixteen on the left. The feet were sore due to slight abrasions but there was no infection.

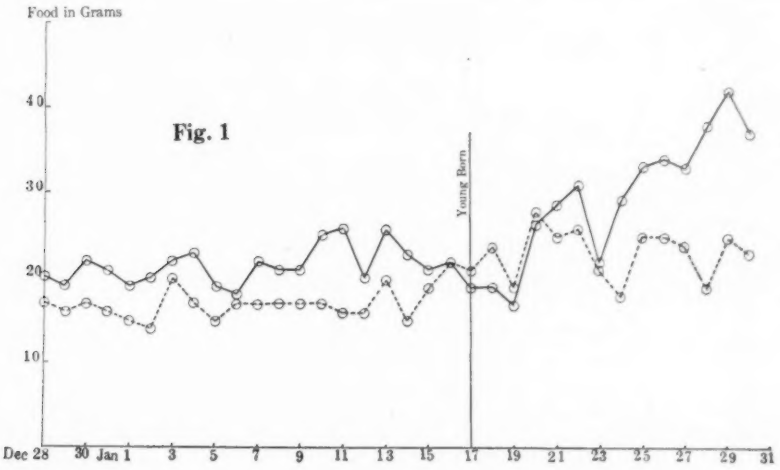

Fig. 1. Graph showing the average daily food consumption of the pregnant rats in continuous line and of the pseudopregnant animals in dotted line during the period of forced work (Dec. 28 to Jan. 15) and for the following fifteen days.

From these conditions we concluded that this animal died from exhaustion. Whether the inability of this rat to withstand the forced exercise given was due to individual variation or to a condition induced by resorption of young we are unable to state. Probably both were instrumental. We further concluded that if fertilization and implantation of several fertilized ova had occurred resorption had taken place to such an extent that only a trace remained.

All of the young born appeared normal excepting the seven delivered by rat 3. These were decidedly underweight and looked poorly nourished. The four males averaged 4.75 grams one day after birth and the three females 4 grams. The males of the other litters averaged, at the same age, 5.4 grams and the females 5.8 grams. These weights correspond with

similar weights of young from normal animals. The young of rat 3 continued under-nourished, apparently due to lack of lactation of the mother. By the fourth day after birth three had died and had been eaten by the mother. The remaining two males and two females averaged 5 grams

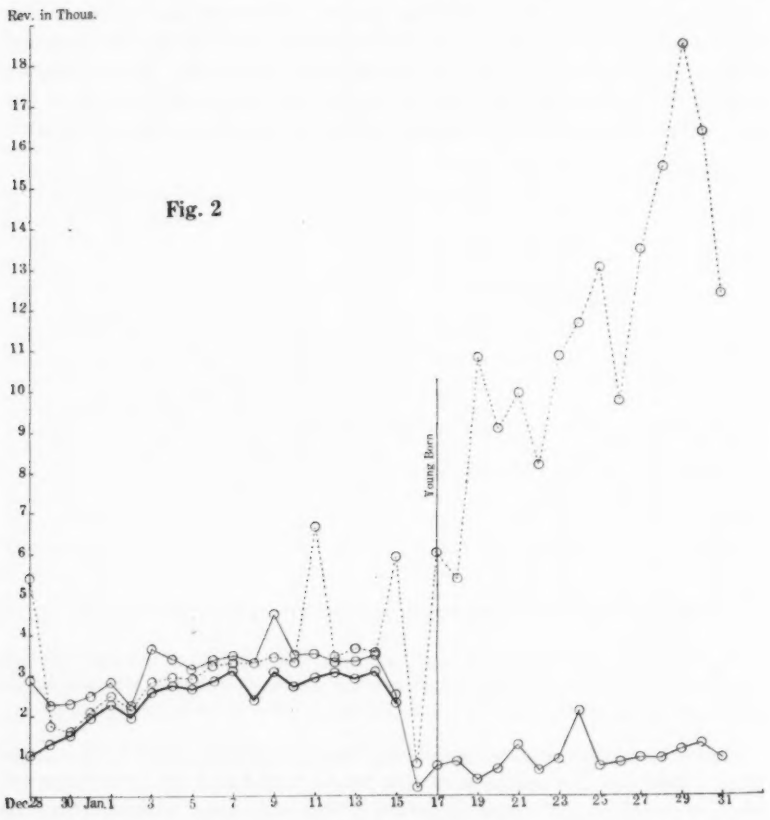

Fig. 2. Graph showing the average revolutions of forced work in heavy continuous line, the total average run of the pregnant rats in light continuous line, and the total average run of pseudopregnant rats in dotted line during the period of forced work and the following fifteen days.

respectively, while the males of the other litters averaged 8.2 grams and the females 7.9 grams. On the eighth day after birth rat 3 had only one young female left which weighed only 4 grams, the others having been eaten. This last one had disappeared by the following day. The average

weight of the other young at eight days was 16.3 for the males and 15.75 grams for the females. The growth of these young compares favorably with our normal growth curves.

We are unable to attribute the cause of insufficient lactation in rat 3 wholly to the forced exercise we gave since we have had occasional cases of a similar kind in our colony. The forced work may have been a contributing factor in lowering the vitality which, in 50 per cent of the individuals, resulted in pseudopregnancy and in rat 3 in a lack of normal lactation.

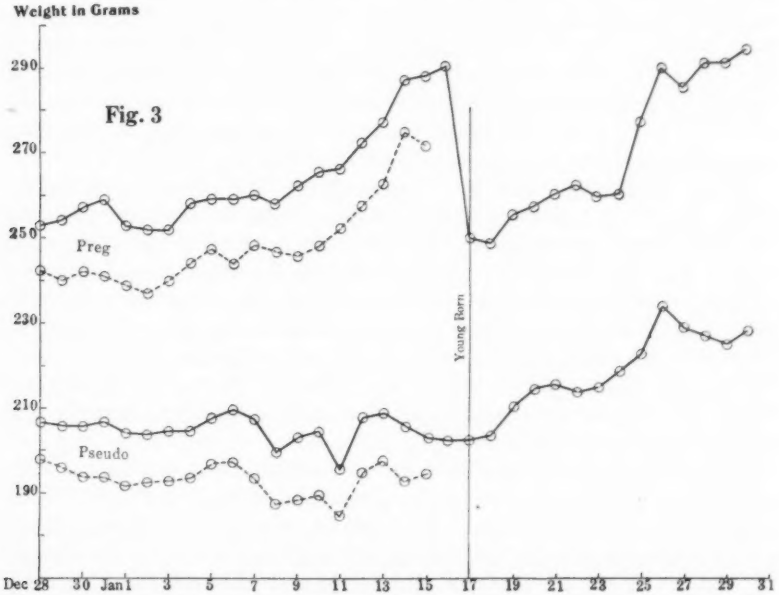

Fig. 3. Graph showing the average daily body weight of pregnant, *Preg.*, and pseudopregnant, *Pseudo*, rats just before forced work and just after cessation of forced work in continuous and dotted lines respectively. The average daily weight of each group is shown for the following fourteen days after forced exercise stopped.

It is interesting to note that all three of the rats, 1, 3 and 7, which had been previously used in experiments 6 and 7 delivered litters and only one of the new rats gave birth to young. This suggests again the value of previous training as mentioned in experiment 7.

None of the litters delivered were large and the general average (6.25) was slightly below that of normal, unexercised rats (6). This suggests that resorption of young may have occurred to some extent. This is especially suggested by rat 7 whose litter consisted of but four young.

When the food consumption of the pregnant and pseudopregnant rats is considered we find that the former ate the most. This is shown in table 17 and in figure 1. By consulting figure 1 it is seen that the average daily food consumption of the pseudopregnant group surpassed that of the pregnant group for only a few days. These days began with the day of delivery of the young. This reduction of food consumption at the time of birth of the young and for a few days following is a normal characteristic of rats, as we have previously shown. The greater food intake of the pregnant group prior to delivery was due to the developing young and to the fact, as shown in table 17 and figure 2, that during this period this group was more active. After birth the difference was due to nursing the young. It is thus seen that nursing the young required a greater expenditure of energy than their inter-uterine development. This is the normal occurrence and not a result of forced exercise. The average daily ratio of food consumption of the two groups is given in table 19.

When the average daily food consumption of the pregnant exercised rats is compared to that of normal pregnant animals in similar cages for the same days of gestation we find that those required to work consumed over 3 per cent more food. This indicates a greater need for energy caused by the imposed work. If a comparison is also made for the same days of lactation we again find that the exercised pregnant rats consumed more food, exceeding the normal controls kept in similar cages by almost 9 per cent. This occurred regardless of the fact that the exercised rats ran a less number of revolutions. The energy turnover however was greater apparently due to recovery.

In figure 2 we have graphically represented the amount of forced exercise given by heavy continuous line, the total average daily run of the pregnant rats by light line and the total average daily revolutions of the pseudopregnant group by dotted line. It is readily seen that the amount of total run in both groups during the period of forced work did not greatly exceed that which they were required to run until about the fifteenth day of pregnancy (January 11) when the pseudopregnant group became much more active. This was due to the reëstablishing of their oestrual cycles about that time. Previous to this time the total activity of the pseudopregnant group was less than that of the pregnant rats. After the reappearance of their oestrual cycles their total run far exceeded that of the pregnant animals. It is interesting to note that the period of least activity of the pseudopregnant rats corresponds to the time when the abnormal process of resorption was occurring and indicates that this was a greater hindrance to activity than the normal development of young.

The amount of forced exercise given in this experiment was sufficient to cause a moderate degree of fatigue by the end of the work period. That the animals were not exhausted is indicated by the amount of voluntary running they did during the remainder of the twenty-four hours. The

animals were apparently able to recuperate and to run voluntarily an average of approximately 800 revolutions before the next work period. The total average daily activity was about 7 per cent below the normal for the same days of gestation. This confirms the statement made earlier in this paper that a revolution of forced work was more fatiguing and required a greater expenditure of energy than a revolution voluntarily turned. After the cessation of forced work there was a noticeable drop in activity in both groups. Following this there was a quick and marked recovery of the non-pregnant rats. The pregnant rats, however, delivered their young two days later after which their curve of voluntary activity as compared to that of normal nursing mothers was very low, being only about 50 per cent normal. Since all conditions except that of forced exercise were the same we are led to conclude that forced exercise during pregnancy reduces the voluntary run during lactation.

When the weights of the two groups are considered we find that the pregnant rats were much heavier and showed a greater gain than the pseudopregnant group. This was due to the fact that the pregnant group had a greater average weight at the time of mating. Three of this group were full grown while all the others had not reached full size. The greater increase in weight was due to the developing young. By examining table 17 and figure 3 it is seen that the pregnant group lost a greater amount in body weight during the periods of forced exercise than the pseudopregnant group. This would be expected since the amount of metabolism is influenced by the size of the animal. If the loss is computed on a percentage basis we find that the average daily loss for the pregnant rats was 6.05 per cent of their weight, while that of the pseudopregnant group was 5.78 per cent. A difference of 0.27 per cent is not great, but nevertheless indicates that the forced exercise was more trying to the pregnant group. Table 17 gives the average number of revolutions turned for each gram lost by the two groups and table 19 gives the daily and average ratios of these results. It is seen that the total average number of revolutions per gram lost by the pseudopregnant rats was 205 and for the pregnant rats 178 revolutions. The pseudopregnant group was thus more efficient and consumed less food than the pregnant group.

The results of this experiment indicate that rather severe forced work is detrimental to normal reproduction, causing pseudopregnancy, death or abortion in 50 per cent of the inseminated animals and a slight lowering in the average size of litters delivered. It also shows that the condition called pseudopregnancy is more trying to the animal and reduces its efficiency more than normal pregnancy. It further shows that the effect of the forced exercise carries over for at least the first twelve days of lactation causing a reduction in voluntary activity and a possible reduction in lactation. And, finally, it suggests a more or less general resorption of the developing young.

SUMMARY

The above experiments seem to warrant the following general conclusions:

1. A revolution of forced activity required a greater expenditure of energy than a revolution voluntarily turned.

2. Forced exercise given early in the day (9 to 4) resulted in a greater additional voluntary run than when it was given in the evening.

3. Forced exercise reduced the normal gain in body weight of pregnant, pseudopregnant and non-pregnant rats.

4. The average food consumption of exercised pregnant rats was greater than that of exercised non-pregnant or pseudopregnant rats.

5. The average daily loss in weight during forced exercise was greater in pregnant than in pseudopregnant or non-pregnant rats.

6. The amount of food eaten was not found to be an accurate measure of the energy turnover as represented by the number of revolutions turned.

7. A close correlation could be made between the weight lost and the revolutions turned.

8. Older rats were less affected by forced exercise than younger animals.

9. The animals were affected by forced exercise in the following descending order: new mothers, pregnant, pseudopregnant and non-pregnant.

10. Animals previously trained to forced exercise were less affected by forced exercise than new untrained rats.

11. Forced exercise during gestation resulted in a reduction in the normal voluntary run of the mothers during at least the first twelve days of lactation.

12. Evidence indicates that forced exercise resulted in cessation of lactation and starvation of the young.

13. Forced work appeared to be the cause of death of several rats during gestation and pseudopregnancy.

14. Death or abortion occurred most frequently about the fifteenth day after coitus which indicates that this is a critical time for the rat.

15. Our general conclusions are that rats given forced exercise during gestation sufficient to cause a marked degree of fatigue tends to cause resorption of young, abortion, pseudopregnancy or death of the adults, a reduction of the number of young born and a lack of normal lactation.

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A STUDY OF TOBACCO SMOKING

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Received for publication March 4, 1925

The widespread use of tobacco has been for many years a subject of curious speculation in respect to either its beneficial or detrimental effects upon the bodily economy. With the development of scientific study of problems relating to hygiene, the question of the effects of tobacco has assumed increasing prominence. Observers are pretty well agreed that distinctly harmful results may follow over-indulgence in tobacco, but the question of just what constitutes over-indulgence has not been answered satisfactorily. So many factors enter into a consideration of the question, such as individual variations in susceptibility, and tolerance, that the task, at present, is extremely difficult, if not hopeless, especially since susceptibility and tolerance are not fully understood.

A review of the investigations of various observers reveals certain facts in regard to the effects of tobacco. Most of the evidence thus far presented shows a lower standard of mental efficiency in tobacco users as compared with non-users of tobacco (1); there is a disturbance of motor reactions in the direction of lowering the efficiency, especially of finely coördinated movements (2); there are slight blood-pressure changes accompanied by vaso-constriction, and in apparently excessive amounts there ensue digestive and visual disturbances and irregularities in the rhythm of the heart (3); whether the toxic effects of tobacco are due to nicotine or some other constituents has called forth considerable investigation, and as a result most observers are inclined to agree that the toxic results are chiefly those due to nicotine and carbon monoxide (4).

The present investigation was undertaken with the idea of determining, if possible, the immediate effects of the use of tobacco. No attempt was made to study the toxic effects or to determine the relative influence of the various factors that might be concerned in the immediate effects. Since tobacco contains substances that may exert drug action, and since most drug habits encountered are those in which the drugs are depressant to the central nervous system, it is conceivable that the tobacco habit might be due to similar effects derived from tobacco, or products derived from it in

[1] Contribution from the Evans Memorial no. 92-D-7.

smoking, chewing or snuffing. Since smoking is apparently the most usual form in which tobacco is used, this method of use was the one chosen in which to determine its immediate effects. If smoking should cause an appreciable depression of the central nervous system, it was thought probable that it could be determined by measuring accurately the effect upon sensation. Therefore, it was decided to study the effects upon a sensory mechanism by measuring the variation in its threshold, if any occurred, with smoking. The sensory mechanism selected was that which is aroused by weak faradic stimulation of the skin of the fingers. If tobacco smoking should cause a depression of sensation it was thought that the immediate effect would be to raise the threshold of the mechanism under observation. Furthermore, it was thought possible that the desire on the part of a person to indulge in a smoke might be the expression of a hyper-irritable central nervous system, in which the consequent demand would be to lessen this hyper-irritability. By repeated satisfaction of this demand by means of smoking, essential foundations are laid for the establishment of a habit. It is a matter of common observation and experience, that smokers in whom the habit is undoubtedly established and who are deprived of their smoke, show unquestionable signs of hyper-irritability of the central nervous system, and that these signs disappear both objectively and subjectively when smoking is indulged in. Most investigative work indicates that the sensory threshold is a reliable index of the state of the central nervous system (5).

Method of study. The Martin method of quantitative faradic stimulation was used throughout this investigation (6). Single break shocks were used, β units being estimated in every instance. Psychical and physiologic factors were carefully controlled. The usual routine of an experiment was as follows. The subject was comfortably seated in a semi-reclining position in an adjustable chair. He faced a blank wall, a blank wall was on his right side, a window was on his left side, but in the semi-reclining position his head was lower than the casement, so that only the sky and tops of trees fell in his line of vision, if it was directed outside. A shade could be drawn, thus excluding the outside view if it was thought to be distracting attention. On one arm of the chair was adjusted a pair of salt solution electrodes, in which the first two fingers of the right hand could rest when the subject's arm was lying comfortably on the arm of the chair. The electrodes were made by cutting off the bottoms of two 2-ounce round glass bottles, and in the neck of each bottle, placing a rubber stopper through which passed a platinum wire, extending about one-eighth of an inch inside the neck of the bottle. The wire inside the bottle was covered with a few drops of clean mercury, outside the wire was connected with copper insulated wires which led to the calibrated induction coil. One per cent salt solution was poured into the electrodes until they were approxi-

mately one-half full. The index finger was immersed in one electrode, the middle finger in the other. The depth of immersion has been shown to be without disturbing effect (7). In the subject's left hand was held a simple spring contact key, in electric circuit with a bell located in the operator's room. The operator began the experiment by throwing in shocks of such strength as had been determined by experience would be below the threshold. Then, gradually increasing strength of shocks was used until the subject by means of the bell, signalled that he had felt the stimulus. Shocks were adequately spaced in order to avoid summation. The subject was always alone in the room when his threshold was determined. The calibrated coil and other apparatus connected with it were in a room distant from the subject, so that sounds connected with manipulation of apparatus were inaudible to the subject. The signalling bell rang loudly enough for the subject to hear it and thus he knew that he had signalled the operator effectively. This was necessary, because in some early experiments in which the subject failed to hear his own signal, the apprehensions of an approaching strong shock were manifested by marked fluctuations of the threshold. After the threshold was first located it was again relocated two or three times, thereby checking for accuracy of the determination. Then three other thresholds were determined and checked as above, in one of which 10,000 ohms resistance, in another, 20,000 ohms resistance, and in the third, 30,000 ohms resistance was placed in the secondary circuit. This latter procedure was necessary in order to obtain data required for calculation of the β units. After the determination of the four thresholds as above described, the tissue resistance of the subject was measured by the Kolrausch method.

The subject was instructed then to withdraw his fingers from the electrodes and begin smoking. Two cigarettes usually were smoked, requiring about twenty minutes. The fingers were then replaced in the electrodes and thresholds and tissue resistance again determined as described above. The whole procedure required from forty-five minutes to one hour. The room in which the subject sat was spacious, and while smoking the doors were open so that adequate ventilation was assured.

The subjects consisted of smokers and non-smokers, both male and female, most of them college students. Because of the necessary training to which subjects were required to submit, it was difficult to maintain attendance of a large number of subjects, for after the novelty of the experiment was passed, the lure of two cigarettes, or the interest in science was not sufficient to induce a return of the subject, especially in the non-smoker, to whom the factor of the cigarette was even less appealing. Consequently, most of the results herein presented were limited to fully trained subjects, although numerous observations which fit in with these observations, were made on those not so well trained. In all, about 750 observa-

tions have been made. Various brands of tobacco were used and while no especially marked variations were observed, yet it was thought best for the sake of uniformity of method, and also for the sake of eliminating variations because of different brands of tobacco, to limit the work mostly to a popular brand of cigarette.

Control experiments were made in which the only variation from the smoking experiments was in the substitution of a period of rest corresponding to the time ordinarily consumed by smoking. Other experiments were made in which the smoking of cubeb cigarettes was substituted for tobacco smoking. It was thought this might show whether the effects of smoking depended upon the specific constituents of tobacco or upon general factors contained in smoke or upon psychical factors.

Results. Martin and his co-workers have shown that the normal sensory threshold, as determined by the Martin method, varies with several factors, notably time of day, also time of week (8). Since our observations were made at various times of the day and week, no definite figure can be given for a normal threshold. In this investigation the term "normal threshold" refers to the threshold or the average of several thresholds determined just before a smoking period or a resting period. The time of day was recorded in each experiment, and the results confirm those obtained by Grabfield and Martin.

In table 1 are shown the typical results obtained with both smokers and non-smokers. The table reveals the general results obtained throughout the investigation. In columns 1 and 2 are shown the subject's habit as to smoking, i.e., smoker or non-smoker, and his normal threshold in β units. In column 3 are shown the thresholds obtained just before smoking, and these are arranged in ascending order for each subject, the first showing a hyper-irritable state, the second an approximately normal state and the third a hypo-irritable condition of the sensory mechanism. In column 4 are shown threshold measurements just after smoking; in column 5 is stated the result upon the sensory mechanism in the way of increased or decreased sensitivity, an increase in a threshold indicating a decrease in sensitivity of the sensory mechanism, and a decreased threshold indicating an increased sensitivity of the sensory mechanism; in column 6 is indicated the percentage change in the threshold as a result of smoking. According to this table the effect of smoking is not always one of depression, but may be that of stimulation to the central nervous system, if the sensory threshold may be taken as indicative of the general state of the central nervous system. The curious fact revealed by the investigation and shown by this representative table is that the effect of smoking seems to be conditioned upon the state of the sensory mechanism at the time when smoking is indulged in. It is noted that when the subject's threshold is definitely below his normal, then smoking causes depression, as is indicated by a rise in threshold,

whereas, when the subject's threshold is definitely above normal, then smoking causes stimulation as indicated by a lowering of the threshold, or in other words, he has become more keen in his detection of a sensory impression. It is important to bear in mind that a low figure in stimulation units indicates keen sensitivity, whereas a high figure indicates dulness of sensitivity.

By reference to the table it is noted that, when the subject's irritability is above normal, the effect of smoking is a marked depression, whereas the

TABLE 1

Threshold variations due to smoking

	NORMAL THRESHOLD	THRESHOLD β BEFORE SMOKING	THRESHOLD β AFTER SMOKING	EFFECT OF SMOKING ON SENSATION	PERCENTAGE CHANGE IN THRESHOLD
	β units	*β units*	*β units*		
Subject B Smoker	196.6	141.0	415.6	Depression	194.0
		196.5	194.5	No change	1.0
		316.3	250.3	Stimulation	20.8
Subject H Smoker	170.7	152.9	250.0	Depression	63.5
		169.2	187.8	No change	10.9
		203.9	152.1	Stimulation	25.4
Subject J. M. Non-smoker	230.5	177.9	294.0	Depression	65.2
		218.0	208.1	No change	4.5
		290.9	137.5	Stimulation	52.7
Subject M. M. Non-smoker	133.1	55.5	119.3	Depression	114.9
		131.5	198.8	Depression	51.0
		223.2	165.4	Stimulation	25.9
Subject M. V. Smoker	127.4	34.8	143.5	Depression	312.0
		144.7	122.9	Stimulation	15.0
		272.1	70.1	Stimulation	74.2
Subject G. K. Non-smoker	92.8	21.0	52.9	Depression	151.9
		74.2	72.1	No change	1.5
		252.0	120.0	Stimulation	52.0

result is one of stimulation when the subject is in a state of depressed irritability. By comparing these results with results in the same subject when he rested instead of smoked, it was seen that rest, if it had any effect at all upon the threshold, was usually in the same direction as that caused by smoking, only much less marked. Any change in threshold less than 15 per cent may be considered no change at all, as the limit of error in this sort of an experiment has that wide a variation. Furthermore it was noted that if the subject's nervous mechanism was at or near normal, then

neither smoking nor resting had a very marked effect. An exception to this is shown in the subject M. M. where a change of 51 per cent was caused by smoking when the threshold was practically normal. This subject from the standpoint of normal threshold was rather irritable, and one would expect a more marked reaction in such a nervous system, when any disturbing factor is introduced. However, excluding this single exception this subject reacted in the same manner as other subjects, with respect to the stimulating or depressing effects of smoking. The results obtained in this subject with cubebs showed stimulation apparently unconditioned by previous state of the sensory mechanism. This was true of all observations with subjects smoking cubeb cigarettes.

In most of the experiments in which rest was substituted for smoking, there was a tendency for the threshold to approach normal if it was previously high or low, and a tendency to remain unchanged if it was previously at or near normal. Smoking had a similar effect only it was much more marked upon a high or low threshold. It is hardly to be expected, however, that the short period of rest, 20 minutes, would produce any great change in the threshold. It is altogether probable that a prolonged period of rest would be necessary to bring back to normal a threshold that was far removed from normal; smoking apparently has the power of bringing the threshold back toward normal in a very short period of time. How lasting the effect of smoking is on the threshold, was not investigated; however, it is conceivable that from common experience of habitual smokers, it is not as lasting an effect as rest, and as the effect wears off, the impulse to smoke begins to rise until it is strong enough to cause the subject again to seek relief by his favorite brand of tobacco. The predominating action of the tobacco is in the way of depression, thus the average percentage increase in the threshold in those instances in which depression of sensitivity occurred, was 72.2 per cent, whereas the average percentage decrease in the threshold in the instances in which stimulation of sensitivity occurred was only 28.3 per cent.

In the experiments in which cubeb cigarettes were substituted for tobacco, they quite generally resulted in stimulation. In only one instance was depression shown and this was so small, 13.1 per cent, that it might easily fall within the limit of error. The effects are not wholly psychical, evidently the tobacco smoke contains something which exerts an action differing from that of cubeb smoke. Whether it is nicotine or not was not determined but the results are rather suggestive, and a fact further suggestive of the nicotine being the active agent is the known action of nicotine on nervous structure, which is one of transient stimulation followed by depression. Depression is the predominating action on nervous structure. In these experiments, depression was the predominating feature. That the volatile products in cubeb smoke are lacking in the elements

that are responsible for the fixing of the habit, is evidenced by the general absence of this type of smoking habit. The stimulation by cubebs is conceivably due to absorption of volatile oil contained therein.

The results obtained with non-smokers did not differ essentially from those of smokers. However, the one instance of greatest stimulation and one instance of greatest depression occurred in a non-smoker. All experiments in which the subject complained of feeling dizzy or sick were discarded. This usually did not happen when cigarettes were smoked but was not uncommon in the experiments in which a pipe or cigar was smoked, and was a complaint not wholly confined to non-smokers. The results herein reported are limited to cigarette smoking. A study of cigar and pipe smoking is being investigated as a separate problem.

Discussion. In connection with a study of the immediate effects of smoking, arises the question of what it is that makes a person desire to smoke at any particular time or what constitutes the stimulus which leads to the consummation of the act and relief from the impelling stimulus. The results obtained in this investigation may aid in a possible explanation of the impulse to indulge in a smoke. Since feeling or sensation is an expression of our physical state, it would be logical to conclude that man feels best when he is in a normal condition. Rest has the effect of causing a return to a normal state and it is common knowledge that many fall into periods of rest so easily that it acquires the character of a habit. Smoking has an effect similar to rest except that it is a much more marked and rapid effect; and if we may look upon resting as a habit, it is not surprising that a more powerful agent such as smoking should engulf a much larger number of people. It should be emphasized, however, that the effects obtained by smoking are much more intense in the way of depression than in the way of stimulation and thus resemble the action of our so-called habit-producing drugs.

The common observation of a man who is nervous, irritable and high-strung, indulging in a smoke to calm his nerves, is readily explained on the basis of the observations made in this investigation. When the nervous system is in a state of hyper-excitability, all incoming stimuli are registered much more strongly and this may be manifested by both physical and mental reactions. Physically, the individual may show varying degrees of restlessness; mentally, he may show a lowering of mental efficiency due possibly to confusion from the strong registration of numerous incoming impulses. A smoke at this time by raising the threshold of the sensory mechanism, shuts out numerous impulses and the central nervous system, relieved of these distracting impulses, enables the individual to become less restless and to think more clearly. On the other hand, the common experience of a mental ennui appearing in brain workers may be relieved subjectively to some extent by smoking, thereby enabling the worker

to "take hold" mentally. This, too, may be explained in the light of the results of this investigation, namely, the stimulating effect produced when the sensory mechanism is in a state of depressed irritability or below normal. These results may also aid partially at least in explaining the fondness for the after-dinner smoke when, as is well known, mental alertness is lessened because of an altered distribution of the blood, then the slight stimulus caused by the smoking and possibly the vaso-constriction, may relieve a mental lethargy that perhaps is approaching annoyance.

In general, it may be said that the commonly experienced stimulating action of smoking upon one who is depressed, and conversely the depressing effect upon one who is irritable, fits in with the above observations in which the threshold for nervous reaction was accurately measured. No explanation is offered as to the factors responsible for this action, since no experiments in which attempts to evaluate the relative influence upon the threshold of rest, nicotine, carbon monoxide, decomposition products and psychical factors were made.

SUMMARY

The results of this investigation seem to warrant the following conclusions.

1. The immediate effect of smoking upon a sensory mechanism is conditioned upon the state of the sensory mechanism at the time when the subject indulges in smoking.
2. The immediate effect of smoking is stimulation when the sensory mechanism is depressed, or depression when the sensory mechanism is in a state of hyper-excitability.
3. The depressing effect of smoking is much more marked upon the threshold than is the stimulating effect.
4. The sensory mechanism of smokers and non-smokers, both male and female, reacts alike to the influence of smoking.
5. Non-nicotine containing smoke does not produce the same results as nicotine containing smoke.
6. The results are suggestive of nicotine action because of the difference of effect produced by nicotine smoke and non-nicotine smoke; and because the known action of nicotine on nervous structure is chiefly that of depression.

The author takes this opportunity to express gratitude to the many subjects who made the investigation possible and for the generous cooperation of Drs. J. P. Bowler, Hanover, N. H., L. F. Hill, Des Moines, Iowa, P. E. Stamatiades, Brushton, N. Y., and to Misses Gladys Kingman and Ruth Connolly.

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DEMONSTRATION OF BRONCHIAL CONSTRICTION AND COMPRESSION FROM PHYSICAL AND OTHER CAUSES

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Received for publication March 4, 1925

Pulmonary distention is a common accompaniment of the anaphylactoid reactions in guinea pigs injected with agar-treated serum, agar sol gel and a variety of other agents. Histologically, the lung changes are characterized by alveolar distention, embolism and thrombosis composed of corpuscles, platelets, fibrin and debris in the pulmonary vessels, hemorrhages, congestion, edema and sometimes perivascular beading, the latter changes being, in part, peribronchial. Bronchoconstriction may be indefinite or not demonstrable in histological sections. That the distention is strictly peripheral and local is indicated by the fact that it occurs on perfusion with inert suspensions of agar. Since the distention of perfused lungs is not prevented or removed by epinephrin, atropin and papaverine, it does not appear to be concerned with direct stimulation of parasympathetic nerve endings and bronchial muscle. Hence the bronchoconstriction, if any, appears to be mechanical. It was on the basis of this evidence that Hanzlik and Karsner (1), in 1920, suggested the nature of the mechanism of the agar action in the lungs. The essential features of the explanation may be restated as follows:

> The agar forms thrombi, which block the small pulmonary vessels surrounding the bronchioles, which in turn are compressed by the *vis-a-tergo* of the pulmonary flow from the right heart of the intact animal, and of the perfusion fluid in surviving lungs. In the perfused lung the flow of perfusion fluid stops. The compressed bronchioles are sufficiently narrowed in calibre to prevent the escape of the air forced in by the violent efforts of inspiratory dyspnea of the intact animal and the artificial respiration of the perfused lungs. In other words, there is a passive bronchoconstriction (really a compression of the bronchi), and as a result permanent inflation of the lung. Since there is no increased excitability of parasympathetic endings or bronchial muscle, it is obvious why atropine and papaverine are ineffective in perfused lungs, and atropine and epinephrine in intact animals. That the effect is purely peripheral, local and mechanical is attested to by the results with lung perfusion, and, on this account, the conclusion is forced that this is the principal and primary mechanism of agar action in intact animals.

Our later studies (2) permit an amplification of this explanation by including the physical influences of edema, congestion and hemorrhages,

which would augment the bronchoconstriction, if anything. Suggestive as the explanation is, and resting as it does principally on indirect evidence, it is not altogether satisfactory without more direct proof of the implied bronchoconstriction. The results which are reported in this paper, it is believed, supply the desired proof.

METHOD. After numerous trials with various procedures, including fresh sections, mass injection of the respiratory tract with celloidin in acetone was resorted to. This method has been employed successfully for the demonstration of blood vessels and capillaries in viscera originally by Krassuskaja (3), later by Huber (4), and recently by Hinman and associates (5). A few preliminary trials indicated that satisfactory and permanent casts of the respiratory tract could be obtained. For my purpose the method had the advantage that immediate precipitation and fixation of the mass occurred and also that it was simple, easy and direct. Moreover the casts were permanent so that a result once obtained could be demonstrated at any time and the results were clear-cut and striking.

The composition of the mass was the same as that used by Hinman and associates for injecting kidneys. The proportion of the ingredients was as follows; celloidin (old x-ray films) 15 grams, camphor 12 grams, and acetone 100 cc. placed in a wide-mouthed bottle. The mixture was shaken from time to time until a thick, translucent and homogeneous solution resulted. Then it was colored blue by the addition of about 1 cc. of a solution of crystal violet and brilliant green, 5 per cent each, in acetone. Coloring with Prussian blue gave casts that were not as elegantly blue as with the dyes. The mixture was left in the original wide-mouthed bottle into which was fitted and wired tightly a rubber stopper with 2 holes. A long glass tube reaching to the bottom of the bottle was inserted in one hole, and a short tube into the other hole. The short tube was joined by a T-tube to a mercury manometer for gauging the pressure and to an outlet for air under pressure, while the long tube immersed in the mass was joined by a short piece of rubber tubing to a short glass cannula for insertion into the trachea. A second T-tube with a screw clamp was attached to the rubber tube joined to the manometer for convenient regulation of the pressure.

As soon as an experiment on a guinea pig was completed the lungs and heart were carefully removed *en masse* together with the trachea as far as the larynx. The organs were transferred to a small bench, the air was started and the celloidin mass forced in until it filled the tracheal cannula about half-way. The cannula was now quickly ligated into the trachea and the air pressure raised at once to 220 mm. of mercury which was the pressure found by trial to fill normal lungs rapidly and completely. The mass injection under this pressure was made for 15 minutes. Then, with the pressure still on, the trachea was ligated close to the end of the cannula, and the injected specimen was transferred to a suitable crystal-

lizing dish and covered with distilled water during the next 24 hours. At the end of this time, the water was removed and the specimen was completely covered with hydrochloric acid (75 per cent of the concentrated acid) during the next 24 hours. The acid digests any tissue, almost completely, and this was then removed, and the specimen restored from a green (in acid) to brilliant blue color, by careful washing with a fine stream of tap water. The casts were kept in distilled water containing a few drops of 40 per cent formaldehyde. They were then photographed to actual size for purposes of reproduction in the accompanying figures.

In the majority of 28 experiments that were performed, guinea pigs of about 300 grams body weight were used. In 2 experiments guinea pigs of 500 and 540 grams were used. In simple injection experiments, the animals were given a whiff of ether and the right jugular vein was quickly exposed. After a short interval the agent was injected intravenously. Ether anesthesia together with artificial respiration were used in experiments in which it was necessary to open the chest for perfusion of the lungs, clamping of pulmonary veins, etc. Pulmonary casts were made from untreated healthy guinea pigs, after injections of peptone, histamine, copper sulphate and agar-treated serum in unsensitized guinea pigs, in anaphylactic shock, after perfusion with washed and agglutinated guinea-pig and beef corpuscles, after pulmonary edema, congestion and hemorrhage.

Controls. Under 220 mm. mercury pressure, the lungs of untreated guinea pigs killed by a blow on the head, and after complete extravasation, i.e., hemorrhage from the carotid and pulmonary arteries, during artificial respiration, were filled completely in about 5 minutes. Illustrations of the controls are presented in figure 1, cast *1*, and in figure 2, cast *6*. The casts of such lungs show the trachea, larger bronchi and lobes, the finer divisions of the bronchi and bronchioles not being discernible, since these are covered by the mass filling the alveoli. On close inspection, the lobes present a fine cellular or honey-combed appearance, but for all practical purposes the lobes appear as solid though discrete masses. This agrees with what is seen as the mass is forced into the lungs, for it appears promptly under the visceral covering, filling the pulmonary surfaces completely. The result is quite different if the bronchi are previously constricted.

Histamine. The suitability of the method for demonstrating bronchoconstriction was tested by the injection of histamine, the most powerful bronchoconstrictor known. That it was found eminently satisfactory is conclusively shown by the striking cast, *3*, in figure 1, obtained from a guinea pig (expt. 1) that received 0.000,000,8 gram histamine per gram body weight (2 cc. of 0.01 per cent) intravenously. Exactly the same results were obtained with two other histaminized guinea pigs. The casts showed a uniform absence of smaller bronchi, bronchioles and alveoli, so

that only casts of the trachea and larger, cartilaginous bronchi remained. This meant that the celloidin mass was prevented from entering the bronchioles and alveoli because of the prevailing constriction of bronchiolar musculature. The bronchioles of guinea pigs are rather muscular and tortuous, and hence favorable to the demonstration of constriction, this condition, in turn, being unfavorable to mass injection, as is indeed clearly indicated by the results obtained.

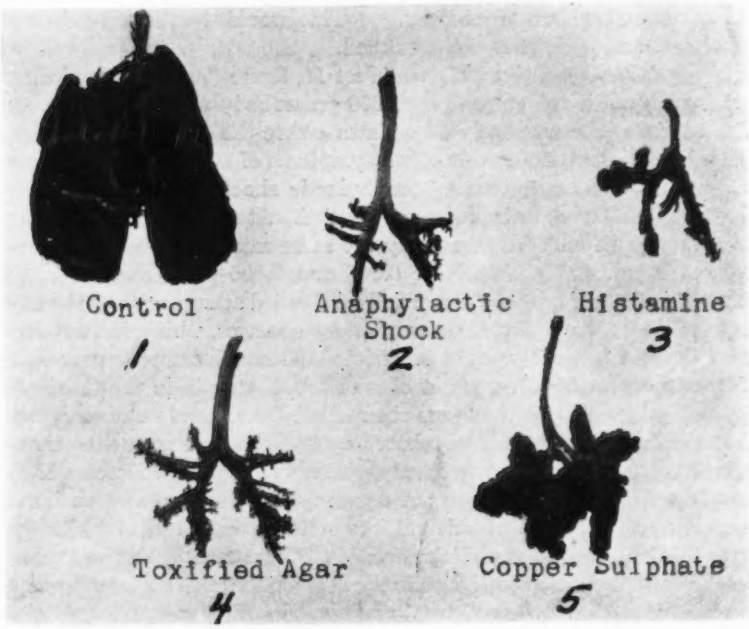

Fig. 1. Casts from guinea pig lungs of untreated control, in anaphylactic shock and after intravenous injections of histamine, toxified agar and copper sulphate. All casts were photographed to actual size.

Anaphylactic shock. If the casts faithfully demonstrate the results of bronchoconstriction, or reduction in bronchial calibre, a histamine result should be obtained in anaphylactic shock. Cast *2*, typifying bronchial constriction of fatal anaphylaxis in guinea pigs sensitized to horse serum, is presented in figure 1, and leaves no doubt of the similarity of the result to that with histamine. Out of five casts obtained from anaphylactized guinea pigs, two were larger than cast *2* in figure 1, indicating better filling of the lungs, owing to less pronounced, or partial, bronchoconstriction. These two guinea pigs showed milder symptoms, and one of them survived.

At autopsy their lungs were only partially distended. Hence the pulmonary casts would be expected to be larger, as was indeed the case. Therefore, there is no doubt that the casts of all the guinea pigs reflected the different degrees of bronchial constriction that supervened. The degree of reduction in bronchial calibre may be roughly conceived of being inversely proportional to the size of the cast obtained. If this is all true, the reduction, or prevention, of bronchial constriction in anaphylactic shock should give larger, or normal, casts according to the degree of bronchial relaxation and filling of the lungs. This would further sustain the correctness and usefulness of the method.

Anaphylactic shock after atropin. Cast *11*, figure 2, shows a typical result from a sensitized guinea pig of 230 grams body weight receiving 0.02 gram atropin subcutaneously 53 minutes before the horse-serum antigen was injected intravenously. Typical symptoms of atropin poisoning were present, but the symptoms of anaphylactic shock, especially dyspnea, were greatly allayed, though the animal died. At autopsy, the lungs were only slightly distended. Essentially the same results were obtained with two other guinea pigs, receiving 0.007 and 0.005 gram atropin. Both animals died, their lungs being partially distended at autopsy and the casts indicated extensive filling of the respiratory passages, which, in turn, indicated that the bronchi were not constricted. Although complete prevention of symptoms and death was not obtained with atropin in the three anaphylactized guinea pigs that were observed, yet the larger pulmonary casts obtained, indicating bronchial relaxation, agreed with the amelioration of pulmonary symptoms and lessened distention. In fact, the casts of the atropinized guinea pigs suggest a degree of bronchial relaxation out of proportion to the prophylactic efficiency of the agent, all of which suggests that the bronchial phenomena are not concerned with the cause of death in anaphylactic shock despite the asphyxia that must supervene as a result of any constriction. Atropin, of course, is known to be an efficient preventive measure in about 70 per cent of guinea pigs under the most favorable conditions. Nevertheless, the method of revealing changes in bronchial calibre by pulmonary casts is seen to be successful in conditions and after drugs known for their effects upon the bronchi, namely, in anaphylactic shock and after histamine and atropin. The method should be useful in detecting bronchial changes from other agents whose effects are not so well understood, and from purely mechanical influences, which probably play a rôle in the pulmonary distention of relatively inert agents. Some of these may now be described.

Peptone. Cast *10*, figure 2, is the result from an unsensitized guinea pig injected intravenously with 3 cc. of 5 per cent peptone (Witte). The animal died at the end of 8 minutes, and the lungs were found markedly distended. The filling with the celloidin mass was incomplete, though

better than in anaphylactic shock. Only the lower lobes showed appreciable filling, the upper lobes being largely free from the mass. In other words, bronchoconstriction was more marked in the upper than in the lower lobes. Irregularity of bronchoconstrictor response is characteristic of peptone action, and, therefore, the result obtained was not at all surprising.

Copper sulphate. This is known to be one of the most powerful agents for eliciting anaphylactoid reactions. Cast *5*, figure 1, was obtained from a guinea pig receiving 0.00013 gram $CuSO_4$ per gram body weight intravenously. Death occurred at the end of two minutes, preceded by the usual symptoms, and at autopsy the lungs were found markedly distended. The pulmonary cast obtained indicated marked bronchoconstriction, being more pronounced than that after peptone, but less than after histamine and in anaphylactic shock. The result agreed with the changes in histological sections previously reported by Hanzlik and Karsner. Besides bronchoconstriction in our previous studies, there were flocculation and complete inhibition of coagulation of shed blood and embolism and thrombosis in the pulmonary vessels together with edema and hemorrhages, suggesting that these latter phenomena were probably concerned with the pulmonary distention and bronchoconstriction and as the later results of this paper will indicate.

Toxified agar. The intravenous injection of serum treated with agar (toxified agar), or of agar sol gel alone in suspension, the agar in either case being in exceedingly small dosage, elicits most violent anaphylactoid reactions and death in unsensitized guinea pigs. In dogs, small doses of agar cause profound circulatory disturbances and other symptomatic and blood changes and sometimes death. Various other changes occurring in guinea pigs have been summarized in the fore-part of this paper. The details of the various changes in both guinea pigs and dogs as well as the method of preparing "toxified agar" have been presented and discussed in previous publications (1), (2), (6). They will be omitted here. It was with toxified agar and agar sol gel that our original explanation of bronchial compression was invoked, and which is being put to direct test in this paper.

Using "toxified agar" prepared from guinea pig serum according to the original directions of Bordet, the following results in three unsensitized guinea pigs were obtained: In the first guinea pig, the usual symptomatic response and death at the end of 3 minutes occurred, and at autopsy the lungs were markedly distended. Cast *4*, figure 1, was obtained from the lungs of this animal, and leaves no doubt of the similarity of the result, namely, marked bronchoconstriction, to that in anaphylactic shock and after histamine. In the second guinea pig, the "toxified" serum that was used stood over night in the ice-chest. No symptoms supervened on

injection, the guinea pig survived, and at autopsy, the lungs were collapsed. A large cast similar to the control (cast *1*, fig. 1) was obtained, because there was no bronchoconstriction, owing probably to inactivity of the preparation used. In the third guinea pig, fresh "toxified agar" gave the usual results, except that death did not occur until the end of 20 minutes and the lungs were partially distended. The cast obtained from this guinea pig resembled cast *5*, figure 1, after copper sulphate. Hence the casts agreed with the responses that were obtained, and faithfully reproduced the degrees of bronchoconstriction that supervened. For the purposes of this study, therefore, it was demonstrated by the method used that bronchoconstriction, or rather reduction in bronchial calibre, occurred in a typical response to "toxified agar," just as was obtained in anaphylactic shock, and after the most powerful bronchoconstrictor known, namely, histamine.

The demonstration of this fact, however, does not as yet indicate the cause of the reduction in bronchial calibre which, as will be recalled from our explanation, was ascribed to compression from the combined influences of several changes (embolism and thrombosis, and we may now add congestion, edema etc., under the *vis a tergo* in the pulmonary artery and of perfusion fluid in perfusion experiments), supervening in the lungs, but not direct stimulation of bronchial muscle to any appreciable degree. A certain amount of muscular contraction from central stimulation set up reflexly from the lungs *in vivo* can not be denied though it has not been demonstrated. All the pulmonary phenomena from agar and other agents causing anaphylactoid reactions having occurred after atropin, epinephrin and papaverine, it is indicated that active contraction of bronchial muscle can be eliminated. Essentially the same conclusion as to absence of bronchiolar muscle stimulation after "toxified agar" was reached by Dale and Kellaway (7) who used different methods. We are left, therefore, with the physical influences mentioned above. The remainder of this paper deals with these physical factors, and the results show clearly that reduction in bronchial calibre can be produced by them individually, let alone their collective influence which is present in vivo after an injection of "toxified agar" or agar and other agents. The various physical influences on bronchi may now be described briefly.

Perfusion of agglutinated corpuscles. Washed, concentrated and agglutinated beef corpuscles were perfused in two guinea pigs, and concentrated, agglutinated guinea-pig corpuscles in one guinea pig. The perfusion was made through the pulmonary artery of lungs in situ, allowing the perfusate to escape from the incised left ventricle, and using artificial respiration for maintenance of respiratory excursions and observation of distention. In experiments of this type it is usually assumed that permanent distention (cessation of spontaneous collapsing), in the absence of

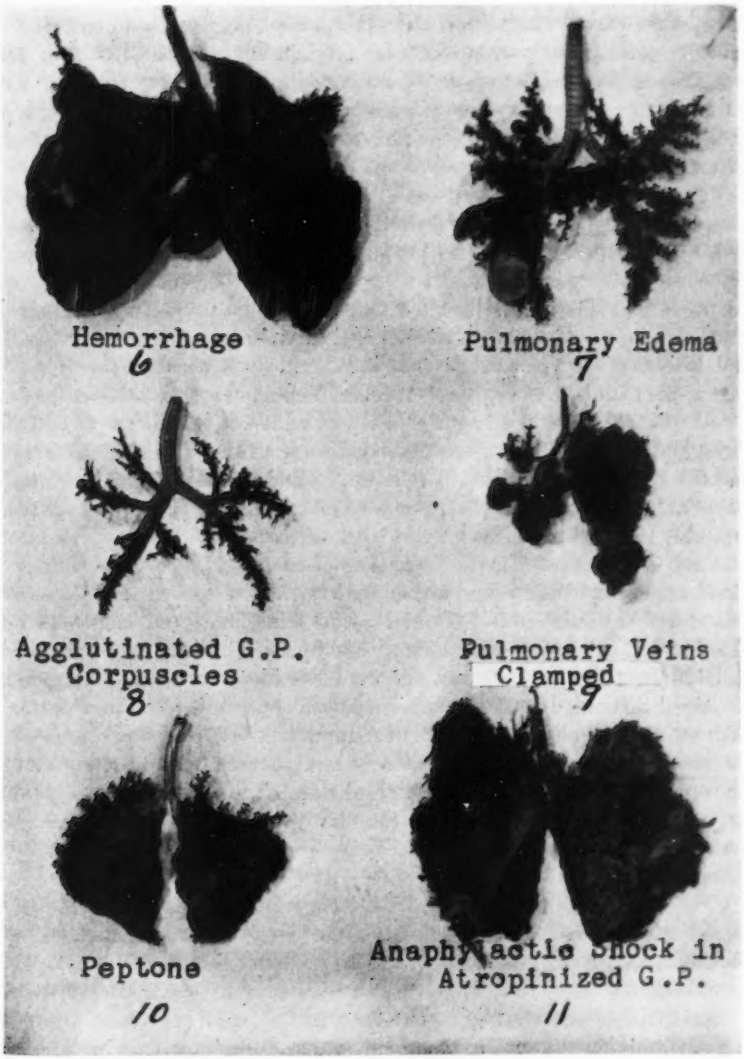

Fig. 2. Casts from guinea pig lungs after marked hemorrhage, pulmonary edema, clamping of pulmonary veins, perfusion of agglutinated corpuscles, anaphylactic shock in atropinized guinea pig, and intravenous injection of peptone. *G.P.* = guinea pig. All casts were photographed to actual size.

edema, means bronchoconstriction as can be shown readily with histamine or peptone. Since beef serum and plasma alone are known to cause bronchoconstriction, the two guinea pigs used in the experiments with beef blood were previously injected with atropin, and during the perfusion atropin and papaverine were injected from time to time into the perfusion tube delivering the agglutinated blood so as to eliminate muscular stimulation from any possible traces of the stimulating constituents of the beef blood. These drugs were not used in the guinea pig perfused with agglutinated guinea-pig blood which is indifferent as to bronchial stimulation in the same species. All the bloods were freshly obtained and washed twice with 0.85 per cent NaCl after removal of the plasma. In consequence of this treatment, the thickened bloods agglutinated spontaneously. The beef blood was used in this thick agglutinated form. The agglutination and viscosity were so marked that the blood flowed rather slowly. The guinea-pig blood did not appear quite as thick and agglutinated as the beef blood. A trace of melted agar sol gel was added to it in order to increase the agglutination. The guinea-pig blood was perfused at a level of 300 mm. and the beef blood at a level of 440 mm. The blood flowed freely from the pulmonary cannula, but the resistance offered by the perfusion apparatus probably reduced the pressure and what this was in the pulmonary artery was not determined. In the beginning of each perfusion, the thickened blood appeared in the left ventricle, but it practically ceased to flow from this chamber at the end of 6 and 9 minutes in the experiments with beef blood and at the end of 4 minutes with guinea-pig blood. At the end of 11 and 18 minutes, the lungs perfused with beef blood ceased to collapse and remained distended, and the same changes supervened in those perfused with guinea-pig blood at the end of 5 minutes. No fluid was observed in the trachea and no edema was grossly recognizable in these experiments. As soon as the lungs were completely distended in each case, the pulmonary arteries were ligated with the fluid still under pressure and the perfusion was stopped. Then the lungs were at once injected with the celloidin mass.

Cast *8*, figure 2, was obtained from the guinea pig perfused with agglutinated guinea-pig blood. The casts after perfusion with beef blood were slightly larger, being about like cast *5* after copper sulphate. They are not reproduced so as to save space. This slight difference is due to experimental error of the method rather than to bronchial relaxation from the atropin and papaverine in these experiments, because loss of muscular tonus in the bronchi would result in diminished resistance, if anything, to the compressing influence of the perfused agglutinated blood. The results obtained, therefore, show that marked reduction in bronchial calibre can be produced mechanically, peripherally and locally in lungs perfused with agglutinated corpuscles of high viscosity.

Clamping of pulmonary veins (congestion). Pulmonary congestion was produced in two experiments by clamping of the pulmonary veins with small serrefine clamps, the thorax being open and artificial respiration kept up until the end of the experiment. The results of experiment 23 on a guinea pig of 310 grams body weight were briefly as follows: Three minutes after clamping of the right pulmonary vein, the corresponding lung remained partially distended, collapsing being present along the edges and around the hilus. At this time the cardiac function appeared to be unchanged, but almost immediately after clamping of the left pulmonary vein, marked dilatation supervened. At the end of 1 minute the heart recovered considerably and continued to beat slowly until the end of 5 minutes, when it stopped. As soon as the heart stopped a clamp was placed on the pulmonary artery. The lower lobes of the left lung were markedly distended, but the upper lobes continued to expand with the artificial respiration and, upon the removal of the lungs, remained collapsed. Small reddish spots, indicating small hemorrhages or markedly congested vessels, were present in both lungs. The lungs were immediately injected with the celloidin mass. Cast *9*, figure 2, shows the result that was obtained in experiment 23. The result of experiment 22 on a guinea pig of 350 grams performed in exactly the same way as experiment 23, was practically the same. The lungs in both experiments did not appear edematous and no edema fluid was present in the tracheas.

There seems to be no doubt, therefore, that marked pulmonary congestion, under the conditions, caused considerable reduction of bronchial calibre, presumably by compression of the bronchioles. The constriction was not as marked as the constrictions in anaphylactic shock, after histamine, and after perfusion of thick, agglutinated blood under pressure, and also during pulmonary edema to be described presently. Whether, or how much, peribronchial and perivascular edema and extravasation of corpuscles were present, can not be said, but grossly, at least, edema and hemorrhages were not demonstrable. If edema and hemorrhages were appreciably present, these changes would undoubtedly contribute to the reduction of bronchial calibre for, as will be shown presently, marked pulmonary edema can bring about just this result, as does also a marked pulmonary hemorrhage. Against the possibility that edema and hemorrhage contributed markedly to the bronchoconstriction in the experiments described, is the rather sudden development of the phenomenon and before the development of gross edema and hemorrhages. At best, under the conditions, therefore, edema and hemorrhage would have a minor rôle in the bronchial compression. Accordingly, the major rôle is left to the sudden and marked congestion.

Pulmonary edema. Pulmonary edema was produced in a guinea pig of 500 grams by perfusion of Tyrode's solution into the pulmonary artery,

after bleeding the animal from the carotid. At the end of 10 minutes, both lungs showed considerable distention, only some local and superficial collapsing being present in the upper lobes. The lower lobes were firm and contained fluid which also was present in the trachea. At the end of 20 minutes there was practically no further change except that more fluid was present in the trachea. The pulmonary edema was marked and the lungs were markedly distended. The experiment was stopped and celloidin mass injected. Cast 7, figure 2, shows the result obtained, and leaves no doubt that reduction in bronchiolar calibre supervened. The result was perhaps better than after clamping of pulmonary veins, but not quite as good as that after perfusion of agglutinated blood and certainly not as good as that after histamine, toxified agar and in shock.

Hemorrhage. Nearly complete removal of blood from the lungs should give the opposite effect of congestion, perfusion and edema, i.e., complete filling of the respiratory tract with the celloidin mass. Cast 6, figure 2, shows the result obtained after complete bleeding of a guinea pig of 540 grams from the right carotid and pulmonary arteries until the heart stopped. This experiment preceded the one on pulmonary edema near the end of the series, and serves, therefore, at the same time, as a further control of the method, operative technique, etc.

Discussion. The method of lung casts employed was found to be satisfactory for demonstrating bronchoconstriction, or reduction in bronchial calibre, under various conditions. Nearly identical results were obtained during direct stimulation of bronchiolar musculature in anaphylactic shock and by histamine and peptone (though less marked), and after agents and conditions, in which stimulation of bronchial muscle had not been demonstrated, namely, after toxified agar and copper sulphate, in marked pulmonary edema, congestion and hemorrhage and after perfusion of agglutinated corpuscles. Hence the purely physical influences of embolism, thrombosis, congestion, edema and hemorrhage in contributing to, or causing reduction of bronchiolar calibre, really bronchiolar compression, after the intravenous injection of such agents as toxified agar, agar sol gel and others, known for such circulatory and blood changes, are emphasized. The results obtained are submitted as direct evidence to support an explanation of the pulmonary changes from agar previously arrived at on the basis of indirect evidence. Whether serum treated with agar, or agar alone, and other inert agents, which cause severe systemic reactions, can stimulate smooth muscle, or if muscular stimulation, however small, is involved in the reactions, is not concerned with the present study. Let it suffice to state that all attempts made thus far to demonstrate stimulation of smooth muscle by toxified agar, agar, etc., have uniformly failed, though this does not mean that it can not occur or that ultimately the conditions under which it may occur may not be discovered.

One may feel optimistic regarding the possibility of this from the recent interesting report of Junkmann (8), who demonstrates direct stimulation of the perfused frog's ventricle by another inert and similar agent, namely, by 1 per cent acacia, with or without calcium, in Ringer's fluid, and antagonism to quinine as a depressant. In any case, the main results from agar and similar agents appear to be explained best on a physical basis.

CONCLUSIONS

1. Changes in bronchi, indicating constriction and compression, were demonstrated by celloidin casts of the respiratory tract in guinea-pig lungs.

2. By this method, nearly identical results were obtained with direct stimulants of bronchial muscle, namely, by histamine and peptone, in the stimulation of anaphylactic shock, and after those agents and conditions which do not demonstrably cause stimulation of bronchial muscle, namely, after toxified agar and copper sulphate and in pulmonary edema, congestion and hemorrhage and in perfusion with agglutinated corpuscles.

3. Hence the physical influences of edema, congestion, hemorrhages, embolism and thrombosis in the reduction of bronchial calibre, really compression of the bronchioles, and occurring after the intravenous injection of toxified agar, agar and other agents are demonstrated. The results are submitted as direct evidence for the mechanism of the pulmonary and bronchial changes previously arrived at on the basis of indirect evidence.

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LESIONS IN THE CEREBRAL CORTEX AND EXTENSION RIGIDITY IN CATS

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Received for publication March 6, 1925

Injury to the motor area of the cerebral cortex of man often results in a condition of permanent contraction of certain muscles, called contracture, hypertonus or spasticity. According to Schäfer's (1900) *Textbook of Physiology*, p. 731, this is not found in other animals besides monkeys, and not always in them. The condition may be due to "the cutting off from the lower centers of the inhibitory impulses which they habitually receive from the cortex cerebri, while excitatory impulses which reach them from the cerebellum are still passing. Hence also it is found in man (and to a less degree in the monkey) that whereas in hemiplegia due to cerebral lesions, both contracture and exaggeration of spinal reflexes is liable to occur, in total transverse lesions of the spinal cord contracture never occurs, and the spinal reflexes are diminished or altogether abolished."

The "decerebrate cat," a preparation which in the hands of Sherrington has yielded such wealth of information in regard to the working of the nervous system, shows to a marked degree the continued contraction of the extensor muscles, or more properly of those muscles which oppose the action of gravity. In this animal influences from the cerebellum are not necessary for the condition of extensor rigidity (Warner and Olmsted, 1923). Furthermore certain reflexes may be exaggerated by spinal transsection, e.g., the flexion reflex (Sherrington and Lawton, 1915). Bazett and Penfield (1922) found that in cats which they had decerebrated by removing a substantial part of the cerebral hemispheres, and which they had kept alive for nearly a month, extensor rigidity manifested itself to a varying degree during the whole period, but that it became less and less constant as time went on, flexion often replacing extension. To produce extensor rigidity in cats it is not necessary to remove the cerebral hemispheres in their entirety; removal of the cortex in the neighborhood of the *sulcus cruciatus* (the motor area) especially ventrally and toward the midline, will produce extensor rigidity. It may be masked by voluntary movements, but when demonstrable it is more pronounced on the side of the body opposite the lesion, if one side of the brain only is injured. Ether rigidity and rigidity caused by removal of this area can be inhibited by

stimulating the ends of the fibers from this area immediately after the injury (Warner and Olmsted, 1923).

This paper deals with the more or less permanent effects of lesion to the cortex in the neighborhood of the *sulcus cruciatus* in cats kept alive after the operation.

The area in question is shown in the left half of figure 1 where the parts are named according to the diagrams and descriptions given in Winkler and Potter's (1914) *Anatomical Guide to Researches on The Cat's Brain.* The *sulcus cruciatus* separates the *gyrus sigmoides posterior*, lying dorsal to it, from the *gyrus sigmoides anterior*, lying ventral to it. In the latter, at the angle formed by it and the *gyrus genualis* is the *area frontalis agranularis.* The cortex around the *sulcus cruciatus* shows similar histological structure to that seen in the motor area of man's cerebral cortex. In the *area frontalis*, as well as in the other areas bounding the *sulcus cruciatus*, an outer layer of small pyramidal cells and an inner layer of large ones can be seen. The fibers from the pyramidal cells enter the white matter of the cerebral hemispheres to form the *corona radiata.* They pass toward the floor of the brain stem in the internal capsule and appear as definite tracts on the ventro-lateral surface of the brain stem, the *pes peduncli cerebri.* In the pons these fibers from the cortex become assorted into a series of distinct bundles, which are separated from each other by the fibers from the pontine nuclei decussating at this point. Winkler and Potter consider that these bundles are divided in the cat as in man, the mesial ones being composed of cortico-pontine fibers, the lateral ones of temporo-pontine fibers, and the remaining central mass of cortico-spinal fibers. Therefore, many of the cortical fibers, especially those bundles in the mesial region from the frontal area, should end here in the pons in association with the basal nuclei, but the majority of them should pass on in the pyramidal tracts through the medulla, where they decussate and pass down into the cord.

Our operations were performed with all the precautions possible to ensure asepsis. The top of the cat's head was shaved and the skin sterilized. The skin and fascia were slit in the midline and the periosteum stripped from the bone. A hole one half inch in diameter was trephined in the skull as far forward as possible without entering the frontal sinuses. Bone wax and hot saline packs were used to stop the hemorrhage. The dura was slit and the surface of the brain exposed. When bleeding ceased the cortex was nicked with a sharp scalpel or scratched with a needle or sharp probe. The wound was closed by sewing together the periosteum, then the fascia, then the skin. The wound was swabbed with iodine and a sterile bandage was fixed in place with collodion and by sewing (fig. 8). In practically every case this wound healed perfectly within a week. The position of the injury was verified at autopsy. In many cases the brains

and cords were preserved for study of the degenerating tracts. The majority of the animals were killed from eleven days to two weeks after this operation. Sections from these were prepared by the Marchi method. Others were allowed to live for a longer period, in some cases for several months. The Pal-Weigert method was used for these.

1. Controls. Two cats were trephined in the usual manner. In one a portion of one *gyrus lateralis* was removed, in the other the most lateral portion of one *gyrus sigmoides posterior*. There was no change in the behaviour of these two cats in any way we could discover. There was no paralysis, no incoördination in walking or in their movements, no spasticity. The brain of the first of these two cats is shown in figure 1. Sections of the brain and cord of the second of these two cats showed a sprinkling of degenerating fibers in one cortico-spinal tract throughout the brain and in the cord. In the pons there appeared to be fewer degenerating fibers in the ventral mesial bundles of one side than in the remaining bundles on that side.

2. Destruction of the whole area about one (the left) sulcus cruciatus. This operation was performed on seven cats and the results were remarkably uniform. As soon as the animals came out of the anesthetic they continued to lie on one side, usually the left. They made no attempt to rise or to use either the fore or hind limb on the right side. There was no evidence whatever of increased extensor tone. Indeed, the exact opposite was the case, for both limbs on the right side were perfectly flaccid. But it was observed that upon lifting these cats off the floor and letting their hind legs hang free for the purpose of comparing knee-jerks (as in fig. 14), the right leg was more or less strongly extended and considerable pressure against the extended foot was necessary to produce flexion. The flaccid paralysis began to disappear by the second day and voluntary movements of the limbs on the right side were then frequent. Most of these seven cats were able to limp about on the second and third days, dragging the right hind limb and doubling the right fore-foot under. They would often fall to the right, the legs giving way under the weight of the body. By the fourth day walking was accomplished in a fairly normal manner, stumbling was less frequent, but jumping from a chair to the floor might cause a fall because of the weakness of the limbs on the right side. At the end of a week one could not have told these cats from normal ones, as far as walking and general behaviour was concerned. They seemed as skilful in washing their faces with the right fore-paw as with the left, and in scratching an ear with the right hind foot as with the left. But upon lifting them up off the floor, we found that in every case the right hind leg, and in some cases the right fore leg as well, was extended. If one pressed with slowly increasing force upon the bottom of the extended foot, the leg would suddenly give way at a certain pressure, a reaction which Sherrington has

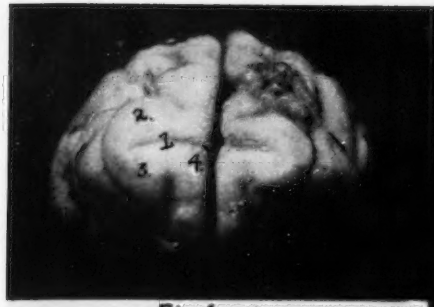

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 1. Brain of cat with cortex destroyed posterior to *sulcus cruciatus*. on one side. No perceptible effect on behavior of cat. *1, sulcus cruciatus; 2, gyrus sigmoidens posterior; 3, gyrus sigmoidens anterior; 4, area frontalis agranularis.*

Fig. 2. Entire area around left s.c. destroyed 9 days before photo taken. Extension of right hind leg.

Fig. 3. Brain of the cat shown in figure 2.

Fig. 4. Entire area around left s.c. destroyed 12 days before photo taken.

Fig. 5. Entire area around left s.c. destroyed 3½ months before photo taken.

called the "lengthening reaction," and which can be shown in decerebrate cats. As soon as the movement of flexion was over, if there was no struggle on the part of the cat, the leg extended once more. A pinch on the toe of the extended foot had to be fairly severe before the cat would flex its leg; on the other side and in normal cats, usually the slightest touch to the toes would cause flexion. Furthermore a pinch on the toes of the left foot caused a vigorous crossed extension reflex in the right leg in addition to the flexion reflex of the left, but the reverse was not true. These phenomena could be observed if the cat could be held with its back on the floor without struggling. The right leg then remained stiffly extended, while the left was limply flexed in response to gravity. When one attempted to make the cat sit up on its hind legs the effect was very striking, for the left hind leg extended as in figures 2, 4, 5. It was sometimes difficult to get the cat to remain in this posture, for it kept backing up in the attempt to flex the right leg.

A nervous, agitated cat would not show these extension effects until calmed by petting and stroking its head. Struggling, kicking and other voluntary movements entirely masked the phenomena. Some cats objected strenuously to being held up by their shoulders, but with patience they could be calmed, and when they ceased to struggle and became rather relaxed and began to purr, then the right hind foot was thrust out in a manner similar to that in a decerebrate cats, while the left was flexed or quite relaxed. The knee jerk on the right side was more brisk, especially when these cats were held up.

The extent of destruction of brain tissue in one of these cats is shown in figure 3. It will be seen that herniation through the trephine hole had occurred in this case, and that adherence to the tissue filling in the hole caused the ragged appearance of the lesion. This brain was removed eleven days after the operation. Two cats were allowed to live for nearly 4 months and throughout this whole period they showed the extension phenomena described above. Figure 5 shows one of these $3\frac{1}{2}$ months after the operation and figure 6 its brain.

The brains and cords of five of these cats were prepared for sectioning. Sections from the brain stem in front of the pons showed practically complete degeneration in one *pes peduncli cerebri*. In the pons all the bundles

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Fig. 6. Brain of cat shown in figure 5.

Fig. 7. Brain of cat with area around one s.c. destroyed, except the *area frontalis*.

Fig. 8. Cat whose brain is shown in figure 7 on the day following the operation. Flaccid paralysis on right side. No extension.

Fig. 9. Same as figure 8. No extension.

Fig. 10. Same cat as in figure 9, the second day after operation. Paralysis disappearing. No extension.

Fig. 11. Same as figure 10.

Fig. 6.

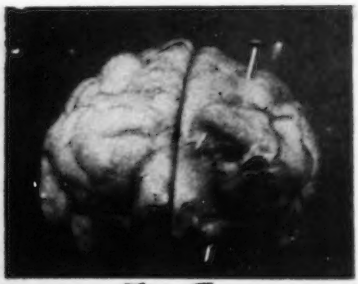

Fig. 7.

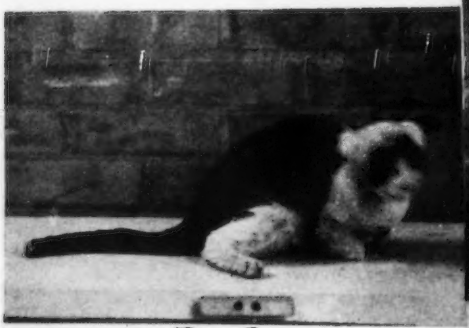

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

of cortical fibers seemed evenly degenerated. In the medulla and cord one cortico-spinal tract was also entirely degenerated.

The results of this type of operation show that the destruction of the cells in the cerebral cortex about one *sulcus cruciatus* causing degeneration in the cortico-spinal tract of one side, results *1*, in temporary flaccid paralysis on the opposite side of the body—walking and ordinary coördinated movements being performed skilfully a week after the operation; and *2*, in extensor rigidity displayed on the side opposite the lesion if the animal is held up or made to sit up on its hind legs.

3. Destruction of the upper and outer margins of the left sulcus cruciatus. Two operations were successful, a third partially so, and the fourth is reported as the second control cat since no effects could be detected. The brain of one of the first two is shown in figure 7, that of the third in figure 12. It will be noticed that in the latter the injury extends further toward the midline, involving the *area frontalis* to a greater degree than in the former. This is correlated with the difference in behaviour of these cats.

All three cats were paralyzed on the right side during the first day (fig. 8). On the next day they were able to limp about, but dragged the right hind foot and often doubled the right fore foot under (fig. 11). Recovery of perfect coördination in walking was complete within a week. The first two cats showed no extension of the limbs on the right side when held up (fig. 10) or made to sit up (fig. 9). The third showed at times evidences of some increase in extensor tone. This was not constant nor marked. The photograph of this cat sitting up is shown in figure 13. The knee jerks of these three cats were at first diminished on the right side. Later no difference could be detected on the two sides.

The brain and cord of each of these three cats was sectioned. Anterior to the pons degenerating fibers appeared scattered uniformly throughout one *pes peduncli cerebri*. At the level of the pons all the bundles of cortical fibers on one side showed degeneration, but those along the ventral border and toward the midline appeared to have slightly fewer degenerating fibers than the other bundles. Degeneration in one cortico-spinal tract was most pronounced in the medulla and cord.

Destroying the cortex on the upper and outer margin of the *sulcus cruciatus* causing degeneration of one cortico-spinal tract results, therefore,

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Fig. 12. Brain of cat with area around one s.c. destroyed with some damage to the *area frontalis*.

Fig. 13. Cat whose brain is shown in figure 12. Paralysis disappeared. Extension not apparent.

Fig. 14. Cat with left *area frontalis* injured. Photo taken the afternoon of the day the operation was performed. Extension of right hind leg.

Fig. 15. Same cat as in figure 14, on the day following the operation. Extension marked.

Fig. 16. Brain of cat two weeks after destruction to left *area frontalis*.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

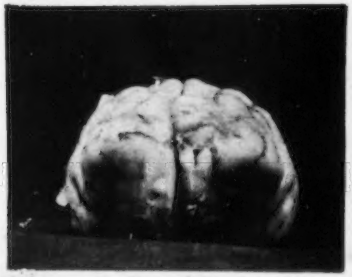

Fig. 16.

in temporary flaccid paralysis on the opposite side of the body. It is evident that the center controlling the extensor phenomena displayed by the cats in the previous section was still present in these cats.

4. Destruction of the margin of the sulcus cruciatus toward the midline on the left side and especially the area frontalis. Six cats were operated upon. None of them showed any signs of paralysis—each was able to walk with perfect coördination upon coming out of the anesthetic. All six, however, showed the characteristic extension of the right hind foot when held up (fig. 14) and made to sit up (fig. 15); they all gave the "lengthening reaction;" the crossed extension reflex was easily elicited in each, and the flexion reflex with difficulty—all the characteristics described in section 2.

All six, however, did not show these characteristics to the same degree; in four cats they were especially pronounced, and in the other two less so. Two of these cats showed extensor rigidity in the right hind limb in an interesting way which was not observed in the others. When they attempted to sit down, and more particularly when they attempted to lower their hind quarters in the act of defecation, the right hind leg instead of flexing, suddenly shot out into the extensor position, and the result was that the cat lost its balance and almost fell over. This occurred several times while we were observing these two cats.

The brains and cords of all six cats were sectioned.

Cat 8

Autopsy. Injury to left cortex in upper margin of s.c. near midline and in *area frontalis.*

Behavior. Extension of right leg (figs. 14, 15). No paralysis, though possible temporary weakness on right side. Knee jerk more active on right side.

Sections. (Weigert.) Slight degeneration throughout one cort.-sp. tract.

Cat 11

Autopsy. Transverse cut 3 mm. long in upper margin of left s.c. next midline.

Behavior. Extension of right hind leg only under appropriate conditions. Difference in knee jerks not marked.

Sections. Died on 6th day. No degeneration apparent.

Cat 12

Autopsy. Transverse cut in *area frontalis* on left side, also nick in center of lower margin of s.c.

Behavior. No paralysis. Extension of right hind leg if cat very calm and contented. No difference in knee jerks.

Sections. Very slight degeneration throughout one cort. sp. tract. In pons all bundles equally affected. Anterior to pons one *pes. peduncli cerebri* has many degenerated fibers.

Cat 102

Autopsy. Upper margin of s.c. destroyed to a distance of 4 mm. from midline on left side.

Behavior. No paralysis. Extension of right hind leg only under the most favorable conditions. Right knee jerks more brisk.

Sections. Very slight degeneration in one cort. sp. tract in cord and medulla. In the pons the ventral and central bundles have fewer degenerated fibers. (Sections anterior to pons lost.)

Cat 104

Autopsy. Slight injury to upper margin of left s.c. more to the *area frontalis.*

Behavior. No paralysis. Extension of right hind leg marked. Little or no difference in knee jerks.

Sections. No degeneration of cort. sp. tract in medulla or cord. In the pons central and dorsal bundles show sprinkling of degenerating fibers, on one side. Anterior to the pons one p.p.c. has large number of degenerating fibers.

Cat 106

Autopsy. See figure 16 for brain.

Behavior. No paralysis. Extension of right hind leg marked. Right knee jerk more brisk.

Sections. Slight degeneration throughout one cort. sp. tract. In the pons all bundles show sprinkling of degenerated fibers on one side, perhaps less numerous in ventral and mesial ones. Anterior to pons one p.p.c. shows large number of degenerated fibers.

This group of cats forms a marked contrast to the preceding group. In the former the true motor area of the cerebral cortex was destroyed, resulting in flaccid paralysis, though temporary; in the latter there was no paralysis, but under appropriate conditions all showed a certain degree of extensor rigidity. It is evident, therefore, that these two nervous mechanisms are separate, and that it is possible so to injure the brain as to remove one and leave the other.

Upon comparing the six records given above it will be evident that the more thorough the destruction of the *area frontalis* the more pronounced the extension phenomena. In every case except one, however, there was clear evidence of degeneration of some cortico-spinal fibers in the cord. But the marked difference in the number of degenerating fibers in the sections anterior to the pons and in those through the medulla shows that many fibers must have come to an end in the pons at the level of the basal nuclei. Judging from our sections one could not say that the fibers from the frontal area are definitely gathered into bundles toward the ventral side of the brain stem. In all cases, no matter where the lesion, it was always the ventral and mesial bundles which showed least signs of degeneration.

5. Destruction of the entire area about the sulcus cruciatus on both sides. Several operations of this type were performed but all except one cat died

within two or three days; the exception lived two weeks. This cat upon coming out from the anesthetic showed extensor rigidity in all four limbs as marked as that seen in many decerebrate preparations. There was no attempt to rise or walk, and there were practically no voluntary movements at first. The degree of rigidity was not constant but varied. On the second day after the operation the cat had recovered sufficiently to stumble about, but it was very weak and unsteady. On the fourth day it was able to walk with a stiff legged gait. It was able to find its food, and ate well. It came when called and responded to petting. It was very restless and until the day before it died it was continually on the move. When it did try to stop, its hind legs remained stiffly extended, hind feet together, and then the animal would circle about to the left using the toes of the hind feet as a pivot. It would keep up this circus movement for minutes at a time crying in a deep voice all the while. It did not appear to be in pain for it cried while evidently experiencing pleasure from petting. When placed on its side it immediately staggered to its feet and started again on its march. Extension was marked when the cat was lifted up or when held on its side on the floor.

6. Destruction of the area frontalis on both sides. Five successful operations of this type were performed. Only one lived more than a week, and this one died through an accident on the 15th day. None of these cats were paralyzed (fig. 17), but all were able to walk upon coming out from the anesthetic. They all cried with the rather hoarse deep cry of the cat described in the previous section. Two walked very little after the first day, but lay on one side, all four legs extended as in decerebrate rigidity, making continuous pawing and scratching motions, the stiffened limbs moving from hip and shoulder. If these two animals became quiet the slightest stimulus would set off the pawing movements and often the crying as well. All these cats showed extensor rigidity upon being held up or made to sit up (fig. 18). The rigidity in these positions was as marked as in decerebrate preparations. Several walked with a peculiar high-stepping gait, lifting the fore feet without bending the knees, as in the goose-step. All were restless and continually on the move. Apparently they were unable to stop even when it was evident by their behavior that they were attempting to sit or lie down. One of these cats in attempting to lower its hind quarters extended both hind legs and sat down suddenly, only to scramble to its feet at once and begin its walking again. Another could not be set down on its feet, for as soon as the hind feet touched the floor it leaped vigorously into the air with a forceful thrust of the extended hind legs; it could only be placed on its side, and then it

Fig. 17. Cat with *area frontalis* on both sides destroyed. Photo taken the morning following the operation. No paralysis.

Fig. 18. Same as in figure 17. Extension in both hind legs.

Fig. 17.

Fig. 18.

kept clawing vigorously, in the attempt to rise. One, a female, exhibited a characteristic sex reflex which involved flexion of the hind legs. Whenever the hair at the base of the tail was touched, even very lightly, the tail flew up and the hind legs were flexed into a squatting position. This position was held only momentarily, the cat hardly stopping in its continuous walk. This reflex was practically indefatigable, for it could be called forth again and again, and in response to the slightest touch.

CONCLUSIONS

1. Cats with the entire area about the *sulcus cruciatus* on one side destroyed develop temporary flaccid paralysis in the limbs on the opposite side. Under appropriate conditions they show extensor rigidity in the limbs on the side opposite the lesion. This extensor rigidity can be shown to be present in cats over three months after the operation.

2. Cats with the upper and outer margins of the *sulcus cruciatus* on one side destroyed show only the temporary flaccid paralysis on the opposite side.

3. Cats with the mesial border of the *sulcus cruciatus* and especially the *area frontalis* on one side destroyed show only the extensor phenomena on the opposite side.

4. Removal of all the area surrounding the *sulcus cruciatus* on both sides results in temporary paralysis, i.e., loss of voluntary movement, and the extension phenomena are very pronounced.

5. If only the frontal areas on both sides are destroyed there is no paralysis, but the extension phenomena are present.

6. Judging from the lesions to the cortex shown at autopsy the more complete the destruction of the *area frontalis* the more pronounced the extension phenomena; injury to the remaining portions of the margins of the *sulcus cruciatus* causes the temporary flaccid paralysis.

7. Judging from the relative number of degenerating fibers in sections of the central nervous system at different levels, the fibers from the frontal areas, loss of which is responsible for the extension phenomena, end at the level of the pons; fibers from the other areas destroyed, resulting in flaccid paralysis, compose the ordinary cortico-spinal tracts.

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THE ASSOCIATION OF COPPER WITH SUBSTANCES CONTAINING THE FAT-SOLUBLE A VITAMIN

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Contribution from the Laboratory of Chemical Research, Kentucky Agricultural Experiment Station

Received for publication March 12, 1925

In a previous report (1) the writer presented data which showed that manganese is closely associated with substances that contain the vitamins. In a further study of the occurrence of appreciable amounts of other elements having catalytic properties with vitamins, data have been obtained which show that copper occurs in the largest amount in those substances that are rich in the fat-soluble A vitamin.

The purpose of this paper is to call attention to the association in nature of small amounts of copper with substances that contain the fat-soluble A vitamin.

The principal sources of the fat-soluble A vitamin are the livers of land and sea animals, butter-fat, yolk of eggs, the germs of cereals, and the young and tender leaves of plants.

Small amounts of copper occur in sea water and in primordial and sedimentary rocks. Fertile soils that result from the disintegration of rocks contain even more copper than the parent rocks, and certain parts of plants contain more copper, when equal weights are considered, than the soil in which they grew. Copper is a normal constituent of plants though it is not distributed uniformly through all the tissues; its concentration is greater in the young and tender leaves than in the older parts, and also greater in the germ of mature seeds than in the endosperm.

From the wide distribution of copper in rocks, soils, plants and in seawater its occurrence in the tissues of land and sea animals is a natural consequence but its concentration in certain organs is of unusual significance. It is a well-known fact that copper is a normal constituent of many marine animals. In certain species of mollusks and crustaceans it has long been regarded as having important biological functions. So far as we are aware, there is no form of plant or animal life in which copper cannot be found in minute amounts whenever sought for by the right methods.

EXPERIMENTAL PART: *Method used in the estimation of copper in animal matter.* The method used in obtaining the results for copper in animal

matter is as follows: 100 grams of finely divided, moisture-free material are transferred to a 2-liter, round-bottom flask, 25 cc. of concentrated sulfuric acid added and the mixture is agitated until the acid is mixed with the animal matter and absorbed. The flask is then placed over a hole on a hot water bath and 10 cc. portions of concentrated nitric acid are added at intervals until the solid particles have been disintegrated and the contents of the flask assume a pasty consistence. This usually requires about 50 cc. of nitric acid. After digestion on the water bath for an hour the contents of the flask are transferred by means of hot distilled water to a 500 cc. flat-bottom procelain dish. The dish is placed on a water bath fitted with porcelain rings and heated until most of the added water has been removed from the contents of the dish by evaporation. The dish is then transferred to a sand-bath and heated gently until the excess of sulfuric acid has been expelled and the contents of the dish have become a dry, solid mass which usually can be broken into small pieces and transferred by means of a platinum spatula to a quartz dish of the proper size and incinerated at a low red heat in a muffle furnace. The resulting ash is then digested in dilute hydrochloric acid and any unburned carbon filtered off, thoroughly washed from chlorides and the process of incineration repeated until the carbon is consumed. The combined solution of the ash is evaporated to dryness in a procelain dish, the residue taken up in about 5 cc. of dilute 1–1 HCl, made to approximately 100 cc. and copper precipitated by passing a stream of hydrogen sulfide gas through the hot solution, which is allowed to stand tightly stoppered until the precipitate has settled. The precipitate is filtered out on an asbestos pad and washed with a cold dilute solution of hydrochloric acid which has been saturated with hydrogen sulfide gas. The copper sulfide is oxidized with nitric acid, evaporated to dryness, the copper nitrate dissolved in water and made to a definite volume. An aliquot is taken and the copper estimated by the Xanthate method (2). At the outset, tests must be made on all the reagents to be used regardless of any statement on the labels concerning their degree of purity.

According to the findings of Maquenne and Demoussy (3), copper occurs in greatest concentration in the young and tender green leaves of plants. It accumulates most in the parts of the plants where there is most water and great vital activity. The parts which gradually dry up and become old gradually lose their copper, yielding it to the growing parts. The mere association of copper in greatest concentration in that part of the plant where there is the greatest vital activity is suggestive of important physiological functions. Herbivorous animals grazing in the open fields always select the young and tenderest shoots and leaves of plants, and thus obtain that part of the plant which contains the largest amount of copper. It is also of interest to note in this connection that

mature dry hays and fodders do not contain as much fat-soluble A vitamin as do the young and tender plants in the green condition. This has been observed in the case of alfalfa in feeding and caring for albino rats. Alfalfa flour which is made by cutting, drying and grinding the young and tenderest shoots of the alfalfa plant has been found to be more satisfactory from the standpoint of vitamin A potency than a similar product made from more mature stalks, branches and leaves of this plant.

The occurrence of copper in greater concentration in the germs of the seeds of plants shows that this element is associated with vitality and incidentally with the fat-soluble A vitamin in another very interesting way in nature. The author has found as much as 20 mgm. of copper per kilogram in dry germs of yellow corn, whereas the starchy and glutinous endosperm contained no more than 0.5 mgm. of copper per kilo of the dry matter.

Apparently the germs of white corn contain less copper (3.5 mgm. per kgm.) than the germs of yellow corn. However, it has not been possible to obtain samples of both yellow and white corn grown on the same type of soil and near each other during the same season. It is contemplated to obtain more definite information on this point during the coming season. The fat-soluble A potency of corn and wheat germs has been recognized for a long time.

Stammers (4) has demonstrated that wheat bran contains the fat-soluble A factor and it has been recognized for some time that the wheat germ is a rich source of this factor. A determination of copper in a sample of wheat germ containing considerable bran, obtained at a local flour mill, gave 58.4 mgm. copper per kilogram of the dry material. A sample of wheat bran obtained at the same mill yielded 16 mgm. of copper per kilogram of the dry matter. The amount of pure germs contained in the mixture of germs and bran probably did not exceed 75 per cent. The result for copper shows that the germ of wheat is much richer in copper than the bran, the sample of the latter being a relatively pure grade of bran.

Copper a normal constituent of milk. Fleurent and Levi (5) report 1.4 mgm. of copper per liter of whole cow's milk, whereas Supplee and Bellis (6) report 0.2 to 0.8 mgm. of copper per liter in freshly drawn cow's milk and an average of 0.52 mgm. of copper per liter for 23 different samples. In the latter case the milk was drawn from the cow into glass vessels and did not come in contact with vessels containing copper.

Hess, Supplee and Bellis (7) analyzed samples of milk of two women for copper and found one to contain 0.4 mgm. and the other 0.61 mgm. of copper per liter.

Steenbock and Sell (8) investigated the proportion of vitamin-A content of skim-milk as compared with whole milk and came to the conclusion

that skim-milk only contains from 20 to 25 per cent of the vitamin-A potency of whole milk.

The following mentioned samples of cow's milk and milk products were obtained at the dairy on the Kentucky Experiment Station farm.

The sample of whole milk was taken from one milking from a herd of about 30 cows. The milk was collected in well tinned vessels and did not come in contact with metals containing copper.

The sample of skim-milk was taken from another portion of the same milking after having passed through a well-tinned separator.

The sample of butter was good quality and consisted of five one-pound molds, which was only a part of a churning made from cream of the Station herd. No salt was added to the butter. As soon as the working and molding of the butter were finished it was taken to the laboratory and transferred to a clean 6-liter glass flask and put into an electric oven regulated at 100°C. where it remained over night. Upon removing the flask containing the melted butter from the oven it had separated into two layers, the clear golden colored fat on top and a residue containing proteid material at the bottom of the flask. The clear hot fat was syphoned into another flask as completely as possible without disturbing the residue at the bottom of the flask. The greater part of the clear fat was separated in this way. The remainder of the fat was separated from the curd by filtering through a pad of paper pulp on a Buchner funnel, suction being used. After washing the residue of proteids with ether to remove the adhering fat the proteid material was dried at 100°C. and ashed at a low temperature in a platinum dish. The dry residue of proteids weighed 42.06 grams and yielded 1.181 grams of ash. Copper was precipitated with hydrogen sulfide from the hydrochloric acid solution of the ash and determined quantitatively. The results for copper are contained in table 1.

Five hundred grams of the clear butterfat were placed in a clean platinum dish and burned by means of an inverted folded filter paper, placed in the dish, which served as a wick. A slight residue of ash remained when the butter fat was thus consumed. This residue of ash was dissolved in dilute hydrochloric acid and tested for copper with hydrogen sulfide. A brownish color was imparted to the solution which showed only a trace of copper was present. There is a probability that some copper was lost by volatilization in burning the butter fat in this way even though a small flame was maintained.

One-half gallon of fresh buttermilk which had been made by churning in an old-style wooden churn with a wooden dasher was obtained and 1500 grams were evaporated to dryness at 100°C. and the copper content of the dried residue determined.

The sample of mare's milk was obtained at the Belmont stock farm and

was collected from about six mares just after the colts were weaned. The milk was collected in glass vessels and strained through clean cheesecloth at the laboratory.

From the foregoing results it is apparent that the greater part of the copper contained in whole milk is removed in the cream when that is separated from the milk, and the greater part of the copper in the cream is retained by the butter when it is churned and prepared in the usual way for food. The occurrence of copper in butter, a substance that contains the fat-soluble vitamin, is suggestive that the copper may have important biological functions in this connection.

Copper in animal tissues. It is a well-known fact that copper is a normal constituent of the tissues and fluids of a large number of species of animals.

Bodansky (9) has determined the copper content of the human brain of four individuals and one fetus. He found from 3.6 to 6.0 mgm. of

TABLE 1

Copper in milk and milk products

MATERIAL ANALYZED	MGM. Cu PER KILOGRAM OF FRESH MATERIAL	MGM. Cu PER KILOGRAM OF DRY MATERIAL
Fresh whole milk	0.38	2.59
Fresh skim milk	0.10	0.98
Fresh unsalted butterfat (free from curd)	Trace	Trace
Curd obtained by melting fresh unsalted butter		5.94
Ash from curd		211.70
Fresh buttermilk	0.13	1.34
Mares' milk	0.30	3.46

copper per kilo of fresh material, in the brains of mature individuals. The fetal brain contained 6.8 mgm. of copper per kilogram.

To further show the presence of copper in normal domestic animals the following samples were obtained at the Kentucky Experiment Station farm, for analysis. The results for copper are shown in table 2.

The first two samples were from a male Jersey calf which was killed 5 days after birth. The next two were from a calf born dead 5 days before the normal time, because of an accident to the mother. This calf appeared to have been normal. The sample of colostrum was from the mother of the same calf. The remaining 5 samples were from a normal steer which had been killed for beef.

From the foregoing results it is to be observed that the tissues of calves at the time of their birth contain relatively large concentrations of copper when compared with the copper content of mature animals. This mobilization of copper in the embryonic stage is undoubtedly of biological significance. It has been shown that a similar mobilization of iron takes

place in the fetus during pregnancy. Bunge offers the following explanation in regard to the mobilization of iron in the fetus. The milk of the mother is low in iron and would not supply the suckling with sufficient iron until it reached the age at which iron could be obtained from the digestion and the assimilation of solid foods. Hence the reserve of iron in the liver. It therefore appears reasonable to assume that this reserve of copper is stored in the liver for a similar purpose during the early stage of the growth of the offspring. The high copper content of the colostrum also tends to indicate a necessary function for this element at a critical stage in the life of the offspring. The source of this copper was in the normal food the mother cow had consumed. It is of interest to note that the blood of the five-day-old calf contained only a slightly larger proportion of copper in the dry material than was found in the blood of a mature ox. The spleen of the mature ox contained a considerably larger propor-

TABLE 2

Copper in the tissues of cattle

MATERIAL ANALYZED	MGM. Cu PER KILOGRAM OF FRESH MATTER	MGM. Cu PER KILOGRAM OF DRY MATTER
Liver of calf 5 days old	100.0	400.0
Blood of same calf	1.6	8.0
Liver of calf born dead	161.3	908.0
Heart of same calf	7.6	36.4
First colostrum from mother of same calf	1.3	5.0
Blood from a mature ox	1.4	7.0
Liver from same ox	12.5	50.0
Pancreas from same ox	1.3	6.5
Spleen from same ox	2.5	16.6
Lean meat from same ox	2.0	4.0

tion of copper than the pancreas. The lean meat of the ox contained a much smaller proportion of copper than any other part analyzed.

Copper content of eggs. Fleurent and Levi (10) obtained 20 mgm. of copper per kilogram in the moisture-free yolk of eggs. The writer obtained only 6 mgm. of copper per kilogram of moisture-free egg yolk, whereas the white of the egg was practically free of copper. These facts establish another interesting coincidence in the association of copper with the fat-soluble A factor.

Table 3 contains the results for copper found in different parts of fowls. The cockerels from which the blood samples were obtained had received the same ration and treatment except that one lot received a very small amount of copper sulfate (1–1400) dissolved in their drinking water for about four weeks previous to the time they were killed. There was some evidence that the birds were benefited to some extent by receiving the

copper sulfate; certainly they had suffered no ill effects from the presence of the copper in their systems.

The sample of turkey blood was obtained at a time when a considerable number of birds were killed for the Christmas market.

The hens' eggs were obtained from the poultry farm of the Kentucky Experiment Station and were produced under normal conditions.

It will be observed that the cockerels which received a small amount of copper sulfate for about four weeks apparently more than doubled the copper content of their blood in the meantime.

Practical poultrymen frequently feed small amounts of copper sulfate at intervals to their birds as an antiseptic for intestinal diseases.

Phillips (11) reports a marked beneficial effect from the use of copper sulfate (1 to 1400 parts of water) in the drinking water for young chickens.

The increased copper content of the blood of the birds to which copper was fed suggests the probability that the birds receiving a normal ration may not be able to obtain sufficient copper for their body needs and when

TABLE 3

Copper in the different parts of fowls

MATERIAL ANALYZED	MGM. Cu PER KILOGRAM OF FRESH MATTER	MGM. Cu PER KILOGRAM OF DRY MATTER
Blood from normal cockerels	1.5	6.09
Blood from cockerels fed $CuSO_4$	3.3	13.30
Blood of normal turkeys	1.5	5.26
Yolk of normal eggs	3.0	6.00
White of normal eggs	Trace	Trace

copper is made available they replenish the deficiency in their blood, thereby becoming more vigorous and more resistant to the diseases to which they are ordinarily susceptible. Further experiments to obtain data on this suggestion are contemplated.

Copper a normal constituent of Albino rats. The Albino rat is widely used for obtaining experimental data in regard to the vitamin-A potency of natural foods and synthetic diets. Insofar as the writer is aware, there are no analyses to show what the copper content is of normal Albino rats. For the purpose of determining whether or not normal Albino rats contain appreciable amounts of copper, the following samples of rats were taken from a colony for analysis. The results of these analyses are contained in table 4. The stock rats are fed the Steenbock ration, supplemented with table scraps, green cabbage, raw potatoes and whole milk.

Twelve rat pups, six each from two litters, of normal size and less than twelve hours old, were chloroformed and the copper content of their bodies determined.

The blood and livers from sixty normal rats that were about 5 months old and whose average weight was approximately 75 grams, were obtained and the copper content determined.

The four guinea pig fetuses analyzed were born dead about a week before the normal time. The mother pig had received a normal diet such as is given to guinea pigs kept for breeding purposes.

From the foregoing results it is apparent that normal Albino rats contain an appreciable amount of copper which may have important biological functions in the metabolism of this animal. It also appears that the rat will absorb several times the normal amount of copper contained in its body when fed a few milligrams of copper sulfate in its food per day, apparently without any ill effects, in the time of this experiment.

It also appears from the foregoing results that the young of Albino rats at birth contain a considerably larger proportion of copper in the dry

TABLE 4

Copper content of albino rats and guinea pigs

MATERIAL ANALYZED	MGM. Cu PER KILOGRAM OF FRESH MATTER	MGM. Cu PER KILOGRAM OF DRY MATTER
Rat pups, 12 hours old	5.4	30.3
Rat livers	5.7	18.8
Rat blood	4.0	9.4
Rat carcasses (minus intestinal tract) average live weight about 100 grams	5.3	16.2
Rat carcasses (minus intestinal tract) live weight about 100 grams + a few mgm. of $CuSO_4$ added to their food per day for about 6 weeks	40.7	124.3
Guinea pig fetuses	4.9	18.6

matter than those of the guinea pig. This can be accounted for by the fact that young guinea pigs are born with teeth and begin eating solid food a few days after their birth whereas the young of rats are born without teeth and several weeks are required before they can consume solid food, hence a reserve of copper is stored in their bodies which is drawn upon until they are large enough to consume solid food.

Copper in the livers of codfish. According to Plimmer (12), fat-soluble A is synthesized in the green parts of plants; lower plants (marine algae) containing chlorophyl synthesize this vitamin. The marine algae are eaten by small marine animals which, in turn, are eaten by larger ones, eventually by codfish. The fat-soluble A factor in the codfish is chiefly concentrated in its liver.

The livers of codfish and the oil prepared from them are regarded as the richest sources of the fat-soluble A vitamin. The tissues of the liver appear to be a richer source of vitamin A than the oil obtained from them.

Apparently, a general impression prevails among the laity and the medical profession as well that the therapeutic value of highly refined, colorless cod-liver oil is less striking than that of the dark or black oils formerly used and which were prepared by the rotting process. Funk (13) states that he has found the crude cod-liver oil to be richer in fat-soluble A vitamin than the highly refined oil. He also states that Zilva and Miura (14) in their experiments with animals found the crude cod-liver oil to have a fat-soluble A potency 250 times greater than that of butterfat.

The following samples of cod-liver products have been examined for their copper content with the results shown in table 5.

The sample of cod-liver chum was obtained from a cod-liver oil factory in the vicinity of Portland, Maine. The chum is the disintegrated liver after it has been subjected to the steaming process. The material was dried at 100°C. It contained some adhering oil.

The samples of refined cod-liver oil were obtained at local drug stores. They had a light golden color. A half dozen or more samples of different brands have been examined by burning the oil in a platinum dish and

TABLE 5

Copper in the livers and oil of codfish

MATERIAL ANALYZED	MGM. Cu PER KILOGRAM OF DRY MATTER
Cod-liver chum	44.0
Cod-liver oil (refined product)	Trace
Coco-vitamin (40 per cent cod-liver oil)	80.17

examining the small residue of ash for copper. In no case has more than a trace of copper been found by this method.

A sample of cod-liver oil preparation labeled "Coco-vitamin" was obtained at a local drug store and copper determined by the method previously described in this paper and the result obtained for copper is given in table 5. The preparation is highly recommended by the manufacturers for its vitamin A and B potency. It is also of interest to note that this preparation contained 4 mgm. of manganese per kilogram of the moisture-free material. The label states that each 100 cc. of the preparation contains 800 units of vitamin A and 54 units of vitamin B.

From the foregoing results it is to be seen that copper is a constituent of cod-livers, very little, however, in the highly refined oil, but considerable in the product coco-vitamin, which contained only 40 per cent of cod-liver oil. This product had a very distinct fish odor whereas the highly refined products had a very faint fish odor.

Affinity of fatty oils for copper. Watson (13), in 1878, studied the action on metallic copper of linseed, olive, colza, almond, seal, sperm,

castor, neatsfoot, sesame and paraffin oil. He found that linseed oil had the greatest action on copper of any of the oils tried, whereas castor oil, paraffin oil, sperm and seal oil had only a very slight solvent action.

To determine if highly purified cod-liver oil and butterfat prepared in the usual way had any solvent action on copper, the following experiments were made. A freshly polished strip of sheet copper was partly immersed in a sample of high-grade cod-liver oil in a test-tube and allowed to remain at room temperature for five days. The cod-liver oil at the beginning of the experiment had a light golden color which was changed to a very pronounced greenish color at the end of the five days. Portions of the greenish colored oil were tested for copper with a solution of potassium ferrocyanide; a brownish red color indicating the presence of copper was thus obtained. Portions of the greenish colored oil mixed with a fresh alcoholic solution of guaiacum produced, upon standing, a bluish color similar to the color obtained when a solution of guaiacum is added to fresh vegetable matter containing oxidases. The blue color was very much intensified upon the addition of hydrogen peroxide and became a deep dark blue on shaking, such as is obtained when a fresh solution of gum guaiac and hydrogen peroxide is added to fresh vegetable matter containing peroxidases. It is a well-known fact that a similar color reaction can be obtained with dilute solutions of pure copper salts.

Another strip of polished sheet copper weighing 3.0210 grams was embedded in a rectangular piece of fresh, unsalted butter weighing 20.85 grams placed in a desiccator at room temperature and allowed to remain there for ten days. When the copper foil was removed it was covered with a deep green film of a copper compound which had been absorbed by the butterfat to a depth of about 1 mm. The strip of copper was cleaned and re-weighed; it had lost 0.0030 gram during the experiment.

It is also a fact that a clean strip of metallic copper will cause a fresh solution of gum guaiac to turn blue, due to catalytic oxidation taking place on the surface of the copper. Apparently certain fatty substances, among which are butter and cod-liver oil, have an affinity for copper.

Discussion. The universal distribution of small amounts of copper in natural waters, rocks, soils, plants and animals is a matter to which but little attention has been given until recent years. The peculiar way in which this element is concentrated in certain important tissues of plants and organs of animals is a problem that attracts interest.

The occurrence of copper in greatest concentration in the vital tissues of growing plants and in the germs of seeds is evidence of important functions in the development and growth of plants.

It has long been known that copper, in the form of hemocyanin, in the blood of certain mollusks and crustaceans has important biological functions in the respiratory process of these animals.

From the results obtained in this investigation it is reasonable to assume that copper is a necessary constituent of the blood of all animal life, including man, and probably performs important functions in the absorption and transfer of oxygen in the respiratory process.

Abderhalden (14) states that in cases of iron therapeutics it should be borne in mind that hemoglobin cannot be formed from iron alone, so that care must be taken to supply the remaining material necessary, in the form of meat, eggs and green vegetables. Undoubtedly such food products are the source of the larger part of the copper found in the animal body.

Its accumulation in greater concentration in the fetuses of mammals is strong evidence that it performs important functions during the development of the embryo and in the early stages of growth after birth. Its occurrence in the yolk of eggs is further evidence of important biological functions in the development of the embryo.

The liver is an organ in which important biological oxidations and transformations are constantly taking place. It has been demonstrated that the tissues of this organ contain oxidizing enzymes, and that it exhibits respiratory properties to a remarkable degree after removal from the animal body, whereas the juices that may be pressed from it have little, if any, respiratory properties. It therefore is reasonable to assume that the copper contained in a normal liver is concerned in the catalytic transfer and oxidative changes that take place in this organ. The fact that the tissue of the liver and the crude black oils prepared from the livers of codfish, have a much greater fat-soluble A potency than the highly refined cod-liver oil is evidence which supports the writer's idea that fat-soluble A is probably a colloidal compound of copper which occurs in the liver of animals to the greatest extent, but which has been eliminated to a large degree in the highly refined cod-liver oil. The same explanation is to be offered in the occurrence of copper in the cream of milk and its subsequent occurrence in butter as it is ordinarily prepared for food.

From the results contained in this and a previous report the author is led to infer that an organic colloidal compound of copper, such as probably exists in green leaves, germs of seeds, whole milk, blood, livers and the yolk of eggs may be the fat-soluble A factor and that a similar compound of manganese in some of these materials may function as the water-soluble B factor.

CONCLUSION

The wide distribution of small amounts of copper in plant and animal tissues generally and its greatest concentration occurring in vital organs leads the author to infer important biological functions for this element in the normal metabolism of plants and animals.

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THE EFFECTS OF EARLY AND LATE BREEDING ON THE MOTHER AND THE SEX RATIO IN THE ALBINO RAT[1]

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Received for publication March 2, 1925

The phenomenon of oestrus or "heat" has for a long time been of great practical interest in animal husbandry and breeding. Many animal breeders believe that the sex of the offspring is greatly influenced by the fact that the female is bred during early or late oestrus. Many obstetricians and gynecologists have also been interested in the study of this subject on lower mammals, since the heat periods and parturition reactions in these animals have a bearing on the more thorough understanding of similar phenomena in human reproduction.

It was in the hope of throwing some light on this subject that this investigation was undertaken. While our efforts have been primarily directed to determining the effect of early and late breeding during oestrus on the sex ratio of the young, other more or less closely related phenomena have been observed and will be briefly referred to in this paper.

To our knowledge, very little or no careful work has been done upon this subject where accurate scientific records have been kept as to the exact time of oestrus that the animals were bred.

R. Pearl (1) reported some work that was carried out in Maine upon cattle. This work was accomplished by means of coöperative breeding records of several cattle breeders throughout the state of Maine. He reported the results of one thousand three hundred and thirteen matings as follows: Those that were bred under three hours after the appearance of oestrus, produced 51 per cent bulls. Those that were bred after three hours and before eight hours from the appearance of oestrus, produced 51.7 per cent bulls. And those that were bred after eight hours from the appearance of oestrus, produced 46.9 per cent bulls. An examination showed that in most cases, the breeding had taken place in the mid-oestrual period. This was undoubtedly due to the lack of scientific and accurate observations by cattle breeders and farmers. They apparently had no means of determining oestrus other than the activity of the cow. This in

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and The Committee for Research on Sex Problems of the National Research Council.

itself is a very uncertain indication of the appearance of oestrus, as the activity of the female varies with different animals. We have found that, in some cases, in the rat, mating would occur from one to five hours before there was any manifestation of oestrual activity. We believe that it is safe to assume that the same is true of the cow. In comparatively small herds of cattle where the bull is allowed to run with the cows, mating probably occurred in early oestrus. On the other hand, where the bull was not allowed to run with the herd, the farmer relied entirely upon the activity of the cow to determine the onset of oestrus. If behavior of the animal was a definite sign of the beginning of oestrus, it would require constant and careful observation. This was probably not carried out by the farmers and breeders. This phenomenon might have occurred during the night when no observations were made. In this case, the breeder's first observation might have been after oestrus had started several hours. In other cases where the owner of the cow did not own his own bull and must take his cow some distance to be served, considerable time would be lost in this manner.

TABLE 1

ANIMAL	TOTAL NUMBER	NUMBER OF MALES TO 100 FEMALES		
		Warm months	Cold months	Whole year
Horse	16,091	96.6	97.3	97.9
Cattle	4,900	114.1	103.0	107.3
Sheep	6,751	102.1	94.0	97.4
Pig	2,357	115.0	109.3	111.8
Wolfhound	17,838	126.3	122.1	118.5

We therefore feel that the lack of scientific observations and tests make these data of little real value. In spite of this, Mr. Pearl concludes that "statistics show no definite or permanent relation between the time in the heat period at which the cow is bred and the sex of the off-spring."

The work of A. S. Parks (2) on the sex ratio in mice was concerned principally with seasonal variation. He does not appear to have kept any accurate records as to the time in the oestrual period at which mating took place.

Different statistical studies have shown that the sex ratio undergoes periodic changes. Miss King (3) has compiled the data in table 1 from the published statements of Wilkins and Heap. This she has verified, under reliable experimental conditions, for the white rat. However, she did not take into consideration the effects of early and late breeding.

Wilckens (5), Bidder (6), Punnet (7) and Copeman and Parsons (8) have collected statistical data from various animals relative to sex, showing that the progeny of young mothers is different from that of older mothers.

Miss King (3) has also supported this with statistics from her own experiments. This is verified, in a more or less general way, by results from our own laboratory, as table 2 shows.

This table is a compilation of some of our past laboratory records. Although there is an apparent exception in litters 7, 8 and 9, there are hardly sufficient data in these litters to draw definite conclusions.

According to Goldschmidt (4) "the natural implication is that the physiological condition of the egg is responsible." He also assumes that there are two different kinds of spermatozoa. He states that there is some evidence indicating that it is possible to explain differences in the sex ratio due to a difference in the behavior of the two kinds of spermatozoa in competition for fertilization. And that it is quite conceivable that the two sorts of spermatozoa have different speeds and therefore different chances of fertilization. He states that certain experiments have been

TABLE 2

SEQUENCE OF LITTERS	NUMBER OF LITTERS IN SERIES	INDIVIDUALS	MALES	FEMALES	NUMBER OF MALES TO 100 FEMALES
1	27	207	113	94	120.1
2	31	232	113	119	94.9
3	16	116	57	59	96.6
4	13	99	53	46	115.2
5	11	72	34	38	89.4
6	8	47	22	25	87.2
7	5	30	15	15	100.0
8	4	25	14	11	127.2
9	3	17	10	7	142.5
10	2	13	5	8	62.5

performed which seem to indicate that it is possible to influence the two kinds of spermatozoa in such a way that a different ability for fertilization can be observed. Stockard and Papanikolau (9) investigated the influence of parental alcoholism on the progeny. They clearly showed in the guinea pig that the progeny from alcoholic fathers were more seriously affected than the progeny from alcoholic mothers. This seems to indicate that the spermatozoa suffered the greater damage. In the progeny of alcoholic fathers, the females showed a greater tendency to die than the males. This was also the case of the progeny of alcoholic paternal grandfathers which suffered even greater damage than the progeny of alcoholic fathers. From this, one might conclude that some definite damage is done to one kind of spermatozoa (in this case, the female producing) more than to the other. From these results, Goldschmidt concludes "that it is possible, by means of definite physiological and pathological agents, to influence one kind of spermatozoa in its power of fertilization more than the other.

This postulates, of course, that there is a kind of fight or competition for fertilization amongst the spermatozoa." This has actually been demonstrated by Cole and Davis (10) in their experiments with rabbits. They mated the same rabbit at the same time with two males of different hereditable nature so that one could determine which was the father by the color of the young. One male showed itself more potent than the other in as much as the majority of the young in the litter always came from him. When the spermatozoa from this same male were damaged through alcoholization, no young were produced by him in the corresponding experiment. They were still produced, however, if this male were used alone for fertilization. The alcoholic sperm were thus capable of fertilization, but were beaten in the competition by the normal spermatozoa.

It is generally conceded by obstetricians that in the human ovulation occurs from fourteen to eighteen days after the appearance of menstruation. They also admit that great variation may occur. Many scientists think that the age of the ovum at the time of fertilization in some way influences the sex. Pryll (17) in regard to this opinion refers to the work of Young and Pflüger who showed that in the frog fresh eggs had a tendency to produce a preponderance of females and as the eggs became older the relative number of males increased. Following up this idea Pryll has tabulated a large number of observations made by himself and some other investigators. A summary of the results is follows:

AUTHOR	NUMBER OF CASES	1-9 DAY		10-14 DAY		15-22 DAY	
		Boys	Girls	Boys	Girls	Boys	Girls
Siegel	80	37	7	4	9	3	20
Sex ratio		528:100		33:100		15:100	
Furst	193	40	18	23	28	27	33
Sex ratio		222:100		82:100		82:100	
Pryll	581	136	111	69	62	71	67
Sex ratio		122:100		111:100		106:100	

This shows a wide variation in the observations of these authors. They all show, however, a reduction in the relative number of males with increasing time after menstruation at which fertilization occurred. Pryll's results, which were based on observations taken during the late war when men were home on short furloughs, show a preponderance of males at all times. He concludes that the comparative statistical method is of little scientific value in solving the complicated problem of sex determination in man.

In this experiment we chose the albino rat as the animal because of its adaptability and ease of control. Thanks to the good work of Long and Evans and to previous activity experiments carried on in this laboratory, we were able to determine almost to the hour the beginning and ending of oestrus in the albino rat and could make matings early or late in heat as desired. We feel that we had in our tests two conditions which might easily modify the vitality of the male and female sex cells in different ways or to different degrees. We have assumed that the age of the egg at the time of fertilization may have a considerable influence upon the offspring. For example, we might assume in the case of the female that is bred during late oestrus, that the ova had been discharged several hours previous to copulation, and had passed a considerable distance down the oviduct, thereby losing some of their vitality. In this case, they would become fertilized with comparatively fresh and probably more vigorous spermatozoa. On the other hand, in the female that is bred during early oestrus, the ova may not be discharged for some time after mating. In this case, the spermatozoa would travel farther up the oviducts and possibly have to wait for the ova before fertilization could take place. The ova then would be fertilized by relatively older and less vigorous spermatozoa. Under these conditions, the sex of the offspring might be affected by assuming that the spermatozoa containing the accessory chromosomes are more or less vigorous than the spermatozoa containing no accessory chromosomes. Thus they might become more or less weakened while traversing up the oviducts and their chances of fertilizing the ova would be affected. The vigor of the fertilized ova might thus be affected and be reflected in the young. Other conditions such as the weight of the young at birth and the length of gestation might also be explained in this manner.

In view of the fact that there are many factors that might influence the sex ratio, we have taken careful precautions to overcome as many of them as possible, so that definite conclusions can be drawn regarding the results of breeding early or late during the oestrual period.

The animals used were all fed our standard diet which has been used for some time in this laboratory and has been found very satisfactory. It appears to contain all of the accessory food factors necessary for health, maintenance, growth and reproduction. The following is the formula for the diet used:

	Grams
Ground whole wheat	67.5
Whole milk powder	10.0
Casein	15.0
Sodium chloride	1.0
Calcium carbonate	1.5
Screened alfalfa	3.0
Butter fat	5.0

The rats were fed upon this diet for one month before the breeding experiment was started. They were all in the best of health. The laboratory used for this experiment was situated upon the third floor of the physiology building and was thus comparatively quiet. It was approximately 45 × 50 feet and was lighted by a skylight of frosted glass approximately 25 × 15 feet. The cages were all covered with black paper to protect the animals from the direct light. The room was ventilated by means of a large electric fan placed in the ceiling so that the air in the room was removed by blowing it to the outside. The temperature was kept fairly constant, varying, as shown by the self-registering thermometer, from 4° to 10°F. daily. Thus the conditions for both early and late breeding were identical in every respect except the time in oestrus at which mating occurred. Any differences in the results therefore could only be attributed to the time of mating.

Twelve young vigorous males were used for breeding throughout the experiment, both for early and late breeding. These males were from four different litters and of approximately the same age. Two of the litters were predominantly females, and two of them were predominantly males. However, in tracing their ancestors back for two generations, there was no marked tendency toward an inherited sex predominance.

The females were of different ages, varying from three months to one year and over. The experiment was begun in early June, 1924, and continued, without interruption, until early in the following December. This length of time we hoped would give an average of any variation due to season. The forty-seven females used were kept in separate cages, fifteen of them in revolving cages, the remainder in stationary cages.

The oestrual period was determined by two methods. The activity of the rats was recorded according to the method of Slonaker (16) who showed that the peak of activity, figure 1, *A*, corresponded to the cornified cell stage of Long and Evans and indicated oestrus. The oestrual cycle of these rats was checked by means of the vaginal smear method described by Long and Evans (11).

For convenience in discussion we have divided the oestrual cycle described by Long and Evans into four stages as follows:

Stage one: This is the same as stage one of Long and Evans. The vaginal smear consists only of medium, uniform sized epithelial cells. The lips of the vagina are somewhat swollen. This stage lasts from ten to fourteen hours. The change to the next stage is characterized by the appearance of a few cornified cells. Mating may occur during the latter part of this stage if especially vigorous males are used. However, since the cornified cells appear very rapidly, indicating stage two, we are inclined to believe that mating will not occur until very early stage two.

Stage two: This is true oestrus and corresponds to stages two and three

of Long and Evans. The vaginal smear consists only of cornified cells. These cells appear rather quickly, within a period of two or three hours. The vagina is open at this time and the lips are swollen having a characteristic purplish color. The oestrual period lasts from twelve to sixteen or sometimes eighteen hours. At the end of this time, the vaginal smear contains large numbers of cornified cells, some of them appearing in large clumps, and the lips of the vagina are less swollen. This latter condition is designated by Long and Evans as stage three. Stage two, as we have used it here, lasts from twenty-four to thirty hours.

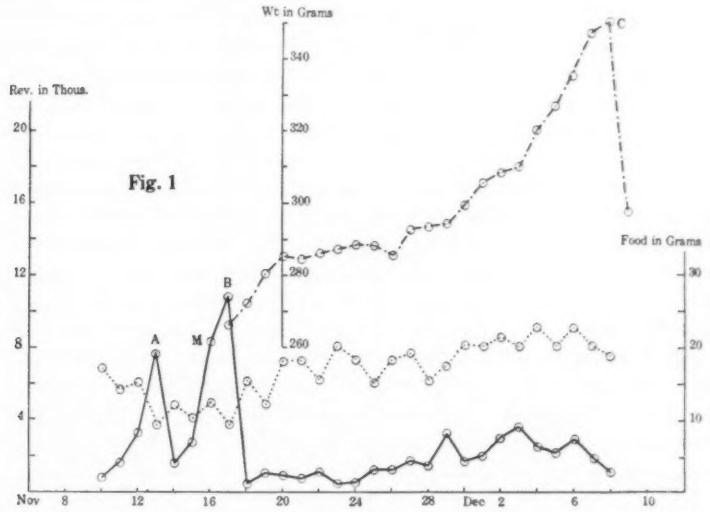

Fig. 1. Represents a typical curve of a pregnant rat which was bred during early oestrus. Dotted line, the daily food consumption in grams. Dotted dash line, the daily weight of the rat in grams. Solid line, the number of revolutions which the rat turned daily. *A-B* represents a typical four-day cycle. The rat was bred at *M* the evening of November 16, the third day after she had reached her last peak. That this was early oestrus, is evidenced by the fact that she ran her customary peak, *B*, after mating had occurred.

Stage three: The vaginal smear contains cornified cells and leucocytes. The lips of the vagina are no longer swollen. This period is relatively short, varying from four to eight hours. It is the same as stage four of Long and Evans.

Stage four: This is the same as stage five of Long and Evans. The vaginal smear consists of leucocytes and epithelial cells. This is known as the dioestrual period and lasts from fifty to sixty hours.

The vaginal smear was taken by the use of a small metal spatula about

3 mm. wide. The procedure was as follows: A drop of normal salt solution was placed upon a glass slide. The spatula, which had been moistened with normal salt solution, was inserted into the vagina, not more than 1.5 cm. and carefully removed while gently scraping the vaginal wall. It was then touched to the drop on the slide. The characteristic cell contents are easily distinguished by means of the low power of the microscope. Care must be taken not to injure the animal as the slightest injury often disturbs the oestrual cycle.

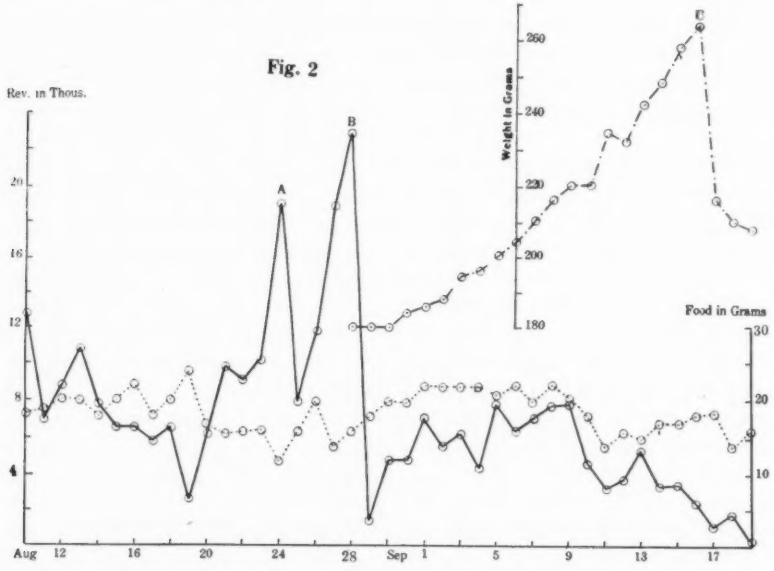

Fig. 2. Represents a typical curve of a rat bred during late oestrus. Dotted line, daily food consumption. Dotted dash line, the daily weight of the rat in grams. Solid line, the number of revolutions which was run daily. The rat was bred in the morning, August 28, at *B*, after she had reached her peak the previous night. The weight curve is a typical curve for a pregnant rat. At *C*, she gave birth to seven young.

The activity cycle, as seen in figure 1, $A = B$, is a direct representation of an oestrual cycle. In the majority of normal, young, healthy females the oestrual periods last approximately four days. However, in older rats, according to Slonaker (16), this may be considerably increased. The activity of the animal was obtained by placing her in a revolving cage with a recording device that recorded each revolution which the animal turned the cage in twenty-four hours. From this, the daily revolutions were plotted. Since the rat is a nocturnal animal, in the majority of

cases, the major part of the running was done during the night. Since there is no pigment in the iris of the rat, it is quite probable that this may be an important reason why they are inactive during the day. Because of their nocturnal habit one would naturally conclude that the oestrual period would begin in the early evening and last throughout the night and the early part of the following day. This was found generally the case with an occasional exception. During the oestrual period, the animal reached the height of her activity or "peak." This was a valuable indicator in determining when the animal was in oestrus, or when she would be in oestrus. In nearly every case, the animal would breed on the morning following her peak the night before, or she would breed on the evening of the third day after her last peak. However, this was not always the case, and it was necessary to check this method by means of vaginal smears. Thus the oestrual period of the animals in the revolving cages could be predicted and vaginal smears were not taken except at the time of mating. The animals in the stationary cages were examined daily by means of vaginal smears in order that the oestrual period might be predicted.

As previously stated, the oestrual period usually began in the evening. We have found it began most frequently between four and ten o'clock p.m. The length of the oestrual period seemed to be closely related to the time of day at which oestrus appeared. For example, if oestrus appeared early in the evening, it usually lasted for sixteen or even eighteen hours. On the other hand, when oestrus appeared late in the evening, it usually lasted about twelve hours or less. However, this was not the only factor which influenced the length of the oestrual period. A young female usually had a longer oestrual period than an older animal. It has also been shown by Slonaker (16) that the oestrual period is influenced to a considerable degree by the diet and physical condition of the rat.

In this experiment we have attempted to breed the females within the last three hours of oestrus for late breeding, and during the first three hours of oestrus for early breeding.

For late breeding we proceeded as follows: The animals in the revolving cages were watched closely and on the mornings after which they had reached their peaks the night previous, vaginal smears were taken. If the smears indicated stage two, consisting entirely of cornified cells, the animal was returned to her cage, and another smear was taken one hour later. If necessary, this was repeated until the smear showed a slight clumping of cornified cells and the number was so increased as to almost entirely fill the field. In nearly every case, when the female was placed with the male she was non-receptive and fought the male for a few minutes. Mating would only take place with an especially vigorous and persistent male. Usually mating would occur only a few times, and the female would become distinctly non-receptive. The female was then removed and

another smear was taken to determine whether insemination had occurred or not. If sperm were present, mating was recorded. It was also noted if a vaginal plug was present. Our experience has shown that the presence of the vaginal plug was essential for fertilization and gestation. In every case, where sperm were found in the vagina after mating, but no vagina plug was present, gestation did not occur. In each case, daily weights were kept of the females. See figure 1 for a typical weight curve for a pregnant rat. It will be noted that the weight increased gradually up to about the fifteenth day after which it increased much more rapidly until the end of gestation. This seems to indicate that at this time a rather sudden change in the rate of growth of the young had taken place.

The oestrual periods of the rats in the stationary cages were predicted and ascertained by taking vaginal smears of them daily. This was done each morning and the oestrual stage was recorded. In this manner stage two, or the oestrual period, could be predicted. When this condition was obtained, the procedure was the same as for the rats in the revolving cages.

In early breeding, the procedure was essentially the same as already described for late breeding. The oestrual periods of the females in the revolving cages could be predicted, and on the evening previous to which they were to run their peaks, which was the third day after they had run their last peaks, vaginal smears were taken and if the smear showed just a few cornified cells, indicating early stage two, the female was placed with the male. Usually the female was not receptive at first, but mating would take place within one or two hours.

The oestrual periods of the rats in the stationary cages were predicted in the same manner as that already described for late breeding.

Vaginal smears were taken after each mating trial to determine whether or not insemination had taken place.

Careful records were kept, where possible, and the exact length of gestation was determined. Immediately after birth the young were weighed and the sexes determined. In those cases where the exact time of birth was not observed, the time could often be approximated by determining whether the young had nursed or not. Since different investigators have shown that the sex ratio is influenced somewhat by succession of litters we have arranged our data in the order of the number of the litter. The results of the first, second and third litters of late breeding are shown in tables 3, 4 and 5.

Table 3 gives the results of the first litters of eleven successful matings during late oestrus. This shows that the average sex ratio of the young for the entire group in this table was 125.5 males to 100 females. This compares quite closely with the sex ratio computed for the first litters in table 2. The average weight of the male young was found to be 4.89 grams, while the females averaged 4.46 grams. The average gestation

TABLE 3

The number, weight and sexes of young of first litters from late breeding of designated mothers

The exact time of gestation is given when it could be obtained. Those marked (?) are only approximate and are not considered in computing the average gestation period.

MOTHER	AGE AT BIRTH OF LITTTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
B-3	156 days	0	0	0	2	11	5.5	22 days 20 (?) hours
B-4	168 days	2	13	6.5	2	13	6.5	22 days 2 (?) hours
C-1	101 days	9	43	4.99	5	22	4.4	21 days 10 hours
C-2	105 days	8	35	4.35	7	28	4.0	21 days 9 hours
C-3	117 days	3	17	5.66	2	9	4.5	21 days 19 hours
C-4	184 days	7	32	4.57	9	35	3.90	21 days 2 hours
C-5	197 days	10	48	4.80	2	8	4.00	21 days 8 hours
D-1	190 days	4	21	5.25	3	15	5.00	22 days 9 hours
D-2	154 days	6	30	5.00	6	27	4.5	21 days 15 hours
D-3	193 days	5	25	5.00	7	33	4.7	21 days 22 hours
D-5	134 days	5	25	5.00	2	9	4.5	22 days 2 hours
Total.....	1699 days	59	289		47	210		
Average..	154.4 days	5.36	4.89		4.27	4.46		21 days 16 hours

TABLE 4

The sex, number and weights of young of second litters of designated mothers from late breeding and the length of the gestation period

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
No. 2	202 days	1	5	5.0	4	20	5.0	22 days 1 (?) hours
No. 21	201 days	5	25	5.0	1	4	4.0	21 days 14 hours
No. 12	202 days	2	10	5.0	5	24	4.8	22 days
D-4	157 days	9	42	4.7	7	31	4.4	21 days 21 hours
No. 7	192 days	5	23	4.6	4	17	4.25	22 days 22 hours
No. 14	194 days	4	16	4.0	5	19	3.8	22 days 7 hours
D-2	186 days	4	21	5.2	2	10	5.0	22 days 20 (?) hours
D-3	230 days	4	20	5.0	5	24	4.8	22 days 2 hours
D-4	187 days	3	17	5.7	4	20	5.0	22 days 2 hours
Total.....		37	179		37	169		
Average..	194.5 days	4.1	4.84		4.1	4.57		22 days

period was 21 days and 16 hours. The average size of the first litters was above the normal average, being almost ten in number.

Table 4, representing second litters from late matings, shows that the average sex ratio of the young in these nine late matings was 100 males to 100 females. This is not greatly different from the ratio of second litters in table 2. The average gestation period was increased about eight hours. An increase was expected since the gestation period increases with age. The average size of the litter was approximately eight and the average weight of the young was almost the same as in the first litter.

Table 5 shows that there was a further increase of males over females in the young of the third litters. The ratio in this case was practically 106

TABLE 5

The sex, number and weights of third litters of designated mothers from late breeding and the length of the gestation period

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
F-1	380 days	2	12	6.0	4	23	5.75	22 days 2 hours
F-2	369 days	5	33	6.5	3	19	6.33	22 days 9 hours
E-3	444 days	1	5	5.0				22 days 7 hours
No. 8	298 days	2	11	5.5	4	22	5.5	22 days 1 hour
No. 10	341 days				1	6	6.0	23 days 1 hour
No. 18	378 days	4	23	5.75	2	10	5.0	22 days 8 hours
F-3	435 days	3	15	5.0	1	4	4.0	22 days 8 (?) hours
No. 6	374 days	1	5	5.0	2	9	4.5	22 days 3 (?) hours
Total.....		18	104		17	93		
Average..	377 days	4.5	5.91		4.25	5.46		22 days 18 hours

males to 100 females. These data also show that there was a great reduction in the average size of the litter with a corresponding increase in the average weight of the young. The gestation period showed a marked increase.

If the average sex ratio from the three litters born from late breeding is computed we find there were 112.8 males to 100 females. There were sixty-five matings made in late oestrus, twenty-eight of which became pregnant as shown in the above tables. The remaining thirty-seven became pseudo-pregnant. Twenty-five of these are shown in table 6. We thus find that 56.9 per cent of the late breedings became pseudo-pregnant.

The females in table 6 were closely observed by their activity cycles and vaginal smears, and the exact time of the reappearance of oestrus was

noted. The exact length of the duration of pseudo-pregnancy was not obtained on the remaining twelve. In this table we have given the ages of the rats, date of mating, date of reëstablishment of oestrus, and the time in days of pseudo-pregnancy. The average length of pseudo-pregnancy was found to be 15.2 days.

We shall not attempt to explain the cause of pseudo-pregnancy, but from observations we wish to offer these suggestions. About the fifteenth day

TABLE 6

Showing the duration of the period of pseudopregnancy in the designated rats mated in late oestrus

RAT	AGE	DATE OF COPULATION	APPEARANCE OF NEXT HEAT PERIOD	DAYS OF PSEUDOPREGNANCY
W-1	367 days	7-20-24	8- 7-24	18 days
No. 4	216 days	7-20-24	8- 5-24	16 days
W-2	368 days	7-21-24	7-28-24	7 days
B-11	247 days	7-22-24	8- 4-24	15 days
W-3	392 days	7-22-24	8- 4-24	13 days
B-4	186 days	7-22-24	8- 7-24	16 days
B-55	247 days	7-22-24	8- 8-24	17 days
W-4	376 days	7-24-24	8- 9-24	16 days
D-4	192 days	7-28-24	8-12-24	14 days
C-1	186 days	7-30-24	8-14-24	15 days
B-3	172 days	7-30-24	8-16-24	17 days
C-5	161 days	7-31-24	8-16-24	16 days
E-2	391 days	8- 2-24	8-17-24	15 days
D-1	152 days	8- 3-24	8-21-24	18 days
D-5	142 days	8-18-24	8-30-24	12 days
E-5	371 days	8- 7-24	8-21-24	14 days
C-2	157 days	8- 9-24	8-28-24	19 days
W-1	385 days	8- 7-24	8-22-24	18 days
W-4	392 days	8- 9-24	8-25-24	16 days
E-2	381 days	8- 6-24	8-20-24	14 days
E-4	372 days	8-10-24	8-28-24	18 days
F-3	362 days	8-14-24	8-31-24	17 days
No. 6	167 days	8-18-24	9- 2-24	15 days
No. 31	154 days	8-20-24	9- 1-24	12 days
F-5	181 days	8-16-24	9- 1-24	16 days

after coitus seems to be a critical period in the gestation of the rat. There is a distinct gain in weight up to this time, as shown in figure 3, weight curve. This might have been due to the animals' lack of activity. It might also indicate that actual germination had taken place, but, for some reason or other, the fetuses were absorbed at about the fifteenth day. However, it seems more probable that coitus produces some sort of inhibitory reaction which produces a delay in ovulation. Later investigation may show what factors are instrumental in producing this condition.

We have given the results of the five litters from early breeding during oestrus in tables 7 to 11.

Table 7 shows that in the first litters of early breeding the total sex ratio of 124.7 males to 100 females corresponds quite closely to the sex ratio of the same litters from late breeding, see table 3. The average length of gestation was twenty-two days which was longer than in late breeding.

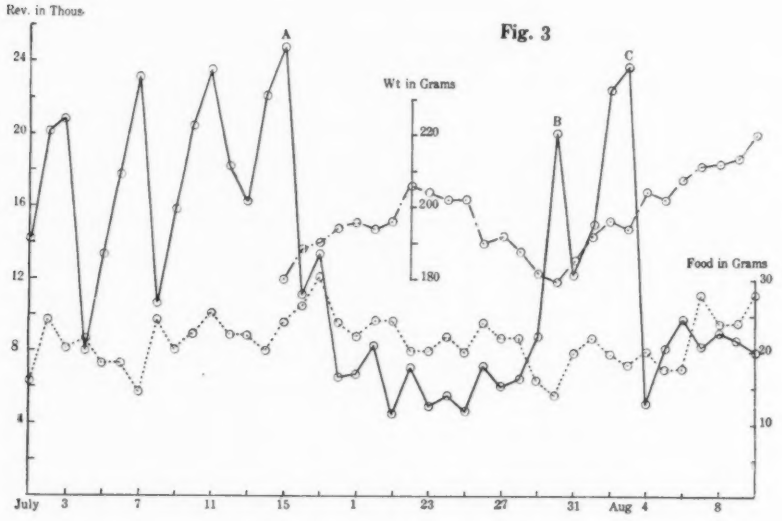

Fig. 3. Graph representing a typical curve of a rat which became pseudopregnant from late breeding. Dotted line, food consumption in grams. Dash-dot line, daily weight in grams. Solid line, revolutions which the animal voluntarily ran daily. The animal was bred at *A* in the morning of July 15 after she had run her peak the night before. From this point to July 28 her activity curve was typical of that of a pregnant rat. At *B*, fifteen days later, she ran another peak, and at *C*, four days later, she was mated again. Notice that after the first mating her weight gradually increased until about the tenth day and then gradually returned to approximately the same as at the time of mating.

In this case, the total sex ratio 104.5 males to 100 females compares very closely to that of table 4. The average period of gestation was twenty-two days and four hours which was again a little longer than in late breeding.

The total average sex ratio in this case, 121 males to 100 females, does not correspond so closely to the same conditions under late breeding. The average gestation period was twenty-two days and five hours.

The total sex ratio was 72.7 males to 100 females. The small number of

TABLE 7

Giving the number, weight and sexes of the first litters of designated mothers from early breeding. The exact length of gestation was noted where possible

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
No. 1	251 days	1	6	6.0				22 days 14 (?) hours
No. 6	282 days	5	20	4.0	4	15	3.66	21 days 20 hours
No. 10	274 days	4	22	4.5	3	15	5.0	22 days 20 hours
No. 51	216 days	2	9	4.5	5	21	4.2	21 days 20 hours
No. 11	260 days	3	13	4.3	4	16	4.0	22 days
No. 17	285 days	5	25	5.0	4	18	4.5	21 days 22 hours
No. 3	261 days	7	34	4.8	1	4	4.0	21 days 15 hours
No. 20	285 days	3	16	5.3	2	10	5.0	21 days 9 hours
No. 2	276 days	3	15	5.0	2	9	4.5	22 days 23 hours
No. 21	261 days	3	14	4.6	4	17	4.25	21 days 20 hours
Total.....		36	174		29	125		
Average..	267 days	3.6	4.83		2.9	4.31		22 days

TABLE 8

The results of the second litters of designated mothers from early breeding

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
B-6	244 days	3	15	5.0	4	19	4.75	22 days 11 hours
D-1	243 days	5	25	5.0	6	30	5.0	21 days 23 hours
No. 41	223 days	1	6	6.0				23 days 1 hour
B-3	245 days	7	31	4.63	2	8	4.0	21 days 20 (?) hours
No. 5	252 days	3	15	5.0	3	15	5.0	22 days 12 (?) hours
C-5	248 days	1	6	6.0	1	5	5.0	22 days 23 hours
C-2	245 days	5	24	4.8	5	24	4.8	22 days
C-1	243 days	5	24	4.8	8	36	4.5	21 days 22 hours
B-1	260 days	4	20	5.0	6	28	4.66	21 days 20 (?) hours
B-4	266 days	6	31	5.17	2	10	5.0	21 days 21 (?) hours
B-2	285 days	3	16	5.33	3	16	5.33	21 days 2 hours
No. 13	264 days	3	15	5.0	4	19	4.75	21 days 20 hours
Total.....		46	228		44	210		
Average..	251.5 days	3.83	4.8		3.66	4.77	4.77	22 days 4 hours

TABLE 9

The results of third litters of designated mothers from early breeding

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
C-4	242 days	3	14	4.66	1	4.5	4.5	21 days 23 hours
C-3	246 days	7	35	5.0	7	32	4.57	22 days 2 hours
D-5	247 days	6	27	6.5	7	28	4.0	22 days
F-3	487 days	5	29	5.8	1	5	5.0	22 days 20 hours
No. 5	246 days	1	5	5.0	1	4.5	4.5	22 days 6 (?) hours
E-3	391 days	1	6	6.0	2	12	6.0	22 days 20 (?) hours
Total.....		23	116		19	86		
Average..	308.5 days	3.83	5.04		3.17	4.52	4.52	22 days 5 hours

TABLE 10

The results of fourth litters of designated mothers from early breeding

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
F-5	487 days	4	20	5.0	3	17	5.66	22 days 20 (?) hours
F-1	482 days				2	12	6.0	23 days 1 hour
F-4	492 days				2	13	6.5	22 days 20 hours
D-3	261 days	2	8	4.0	3	11	3.66	22 days 21 hours
Total.....		6	28		10	53		
Average..	431 days	1.5	4.66		2.5	5.3		22 days 22 hours

TABLE 11

The results of fifth litters of designated mothers from early breeding

MOTHER	AGE AT BIRTH OF LITTER	NUMBER OF MALES	TOTAL WEIGHT	AVERAGE WEIGHT	NUMBER OF FEMALES	TOTAL WEIGHT	AVERAGE WEIGHT	GESTATION
			grams	*grams*		*grams*	*grams*	
D-4	260 days	4	24	6.0	2	11	5.5	23 days (?) hours
D-2	280 days	2	13	6.5	2	11	5.5	22 days 16 hours
Total.....		6	37		4	22		
Average..	270 days	3	6.12		2	5.5		

TABLE 12

Showing average results of late and early mating during oestrus

LITTER	LATE BREEDING										EARLY BREEDING									
	Average age of mothers	Young					Average gestation period	Number of matings	Per cent pregnant	Per cent pseudopregnant	Average age of mothers	Young					Average gestation period	Number of matings	Per cent pregnant	Per cent pseudopregnant
		Males		Females		Number of males per 100 females						Males		Females		Number of males per 100 females				
		Average number	Average weight	Average number	Average weight							Average number	Average weight	Average number	Average weight					
1	154	5.4	4.9	4.3	4.5	125.5	21 days 16 hours				267	3.6	4.8	2.9	4.30	124.7	22 days 0 hour			
2	195	4.1	4.8	4.1	4.6	100.0	22 days 0 hour				251	3.8	5.0	3.6	4.77	104.5	22 days 4 hours			
3	377	2.25	5.9	2.14	5.5	106.0	22 days 18 hours				308	3.8	5.0	3.3	4.52	121.0	22 days 5 hours			
4											431	1.5	4.7	2.2	5.30	72.7	22 days 22 hours			
5											270	3.0	6.0	2.0	5.50	150.0	23 days 0 hours			
Average.	243	3.91	5.1	3.51	4.9	110.5	22 days 3 hours	65	43	57	310	3.1	5.1	2.8	4.8	114.6	22 days 11 hours	72	67	33

observations and the great variation of rats probably accounts for this variation in sex ratio. The average length of gestation was twenty-two days and twenty-two hours.

The total sex ratio, in this case was 150 males to 100 females. The exact period of gestation was not obtained for rat D-4, but it was relatively long, making the average gestation period for this group approximately twenty-three days.

One rat, F-2, age 486 days, gave birth to her seventh litter of five males and one female, a sex ratio of 500 males to 100 females.

This concludes the litters from early breeding. There was a total of seventy-two matings, forty-eight of which became pregnant and 33⅓ per cent resulted in pseudo-pregnancy. Two of the females ate their litters before the number and sex could be determined, and one female died while pregnant.

In order to make an easier comparison between the results of late and early breeding as shown in the foregoing tables we have put the average results into tabular form in table 12. These results show that the sex ratio of the young from early breeding showed a somewhat greater proportion of males than in late breeding. The difference however was so slight as not to warrant any definite conclusions. The results also seem to indicate that early breeding was less favorable to large litters though the average size of the young was not affected. Early breeding also seems to have increased the gestation period. This we would expect for if insemination occurred early, doubtless some time before ovulation, there would be a delay in fertilization and the beginning of gestation. Since we have always computed the gestation period from the hour of successful coitus the lengthening of eight hours could be accounted for.

It can be seen from the above tables of both early and late breeding that the gestation period became progressively longer as the age increased and with successive litters. The average gestation period for young rats from three to six months of age was found to be slightly over twenty-one days. It was found to be very close to twenty-three days for rats from fourteen to eighteen months of age.

CONCLUSIONS

From the above, we have been able to draw some rather definite conclusions:

1. Breeding early or late in the oestrual period, had only a slight influence upon the sex ratio of the young. There was a small increase in the number of males in early breeding.

2. The per cent of pregnancy was greatly influenced by the time in oestrus that the female was bred. In late breeding, pseudo-pregnancy

occurred in 57 per cent and in early breeding 33 per cent of the cases in which insemination took place.

3. Females were most receptive during the first three hours of oestrus.

4. In the great majority of cases, the oestrual period began between four and ten o'clock p.m.

5. The changes in the cellular content of the vagina took place within an exceptionally short time at the beginning of oestrus.

6. There was no marked difference in the size of litters or size of young in litters from early and late breeding.

7. The oestrual periods of our albino rats varied from twelve to eighteen hours.

8. Our observations showed that the vaginal plug seemed to be essential for fertilization and gestation.

9. The length of the gestation period increased with successive litters and the age of the rat.

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FURTHER EXPERIMENTS ON THE ACTION OF INSULIN

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Received for publication March 6, 1925

In seeking to explain the mechanism of the action of insulin on glucose we have one chief fact to guide us, viz., that insulin causes sugar to disappear more or less completely from the blood, and that this is not due to its excretion by the kidney. It has been shown both in this laboratory and elsewhere that this is not due to blood glycolysis (1). We are thus driven to assume that the glucose disappears into the tissues. It appears as if the sugar in the blood were in equilibrium with something in the tissues, possibly free sugar, and this in turn with another substance, perhaps reserve, perhaps a substance forming a stage of the intermediate metabolism, and that insulin decreases the amount of one of these present, upsetting the equilibrium all the way along in such a direction as to cause sugar to move from the blood to the tissues. The first thought that occurs is that the oxidation of glucose is increased, thus lowering its concentration in the tissues. Although in certain animals as the dog and cat both the respiratory quotient and the oxygen consumption may increase following insulin, this is insufficient to account for the sugar that disappears (2). We then turn to glycogen, but here it has been clearly established that the conversion of sugar into glycogen is not promoted by insulin (3). Since the amounts of glucose that can be made to disappear from the blood stream by insulin are very great, and since this glucose is neither oxidized nor converted into glycogen, we conclude that it disappears either as a carbohydrate which is not glycogen or that it is changed into something of a non-carbohydrate nature. In this paper we report some experiments bearing on the first of these possibilities.

I. THE SOLUBLE CARBOHYDRATES OF LIVER AND MUSCLE. Although the work of numerous investigators (Bernard, Pavy, Seegen, Kleiner, Palmer (4) etc.) indicates that very great technical difficulties stand in the way of an accurate determination of the sugar and polymerized carbohydrates of the solid tissues it seems clear that the only way by which the possibility of the formation of such compounds as these referred to above can be settled is by making such determinations. The present is a preliminary communication of the results so far obtained by us in this direction.

Methods. The general plan has been to compare the reducing power of extracts of liver and muscle prepared from normal animals with those prepared from animals injected with insulin. This has been done before and after hydrolysis of the extracts by heating with weak mineral acid. The results before hydrolysis are taken to represent the free reducing sugars, and those following it, polymerized carbohydrate, or some complex containing sugar groups.

Since, as is well known, glycogen begins to be hydrolysed to form glucose almost immediately after death—particularly in the liver (*post-mortem* glycogenolysis)—the tissues were rapidly removed after stunning the animals (rabbits), before the heart had ceased to beat, immediately frozen by liquid air and crushed into small pieces in an iron mortar. Portions of the frozen material were then rapidly weighed in flasks and the extracts prepared by one or other of two methods which were as follows:

1. Mixing with enough 95 per cent alcohol to give a concentration of 70 per cent and then immediately heating on the water bath. After decanting and filtering the extract the residue was ground in a mortar and reëxtracted with 70 per cent alcohol several times.

2. Mixing with alcohol which had previously been cooled to below 0°C. This was done in a mortar surrounded by freezing mixture and in this the frozen tissue was cut up and crushed by strong bone forceps and a pestle. The extract was decanted into large tubes and rapidly centrifuged and the *clear* supernatant fluid poured off into flasks. The residue in the mortar and the sediment in the tubes were repeatedly extracted, great care being taken that nothing but clear extract was collected in the flasks.

The extracts were evaporated either on the water bath, or, in the case of those prepared at low temperature, in a current of warmed air. After being brought just to dryness, the residue was made up to a round volume, with water and an aliquot portion hydrolysed with 2 per cent hydrochloric acid. To both hydrolysed and unhydrolysed portions, phosphotungstic acid was added till no more precipitate formed and after standing 24 hours, the sugar was determined by the Shaffer-Hartmann method, either directly or after removal of the phosphotungstic acid by barium chloride.

Results. In certain of the experiments difficulties arose which rendered the results undependable. The others are recorded in table 1. The following results are of interest:

1. In normal animals hot alcohol extracted somewhat less reducing substance from muscle and distinctly less from liver, than did ice-cold alcohol. This may indicate that heat either destroys some of the reducing substance, or changes it, perhaps by causing it to combine with some other material, so that it is not extracted.

2. With one exception hydrolysis caused a greater increase in reducing power in extracts of muscle made with hot alcohol than in those made with

chilled alcohol. In the latter, indeed, hydrolysis sometimes caused a diminution in reducing power. The effects of hydrolysis on the liver extracts by both methods were very irregular, causing sometimes an increase and sometimes a decrease of reducing power. The latter was

TABLE 1

Milligrams sugar per 100 grams of original tissue

NUMBER	METHOD	MUSCLE					LIVER					BLOOD SUGAR PER 100 CC.
		Before hydrolysis	Average	After hydrolysis	Average	Difference	Before hydrolysis	Average	After hydrolysis	Average	Difference	
					Part 1							
					Insulin							
												mgm.
1	70 per cent hot alcohol.....											
2	70 per cent hot alcohol.....											
3	70 per cent hot alcohol....	50		94			129		181			
		42	46	72	83	37	—	129	145	163	34	
4	70 per cent hot alcohol....	17		170			189		244			
		33	25	220	195	170	220	205	282	263	58	
5	70 per cent hot alcohol....	—		—			106		191			
		88	88	100	100	12	115	110	197	194	84	
Average.....			53		126	73		148		207	59	
					No insulin							
1	.....	91		—			177		145			
		66	79	128	128	49	181	179	181	163	−16	
2	.....	67		100			168		148			
		94	80	115	108	28	231	200	201	175	−25	
3												
4	.....	73		281			256		331			
		85	79	269	275	196	197	226	431	381	155	
5	.....	81		123			197		256			
		42	61	146	135	72	192	195	254	255	60	
Average.....			75		162	86		200		244	48	

TABLE 1—*Continued*

NUMBER	METHOD	MUSCLE					LIVER					BLOOD SUGAR PER 100 CC.
		Before hydrolysis	Average	After hydrolysis	Average	Difference	Before hydrolysis	Average	After hydrolysis	Average	Difference	
					Part 2							
					Insulin + glucose							
												mgm.
9	Iced alcohol											
10	Iced alcohol											
11	Iced alcohol	184		187			387		811			
		178	181	202	194	13	301	344	774	792	448	
12												
13	Iced alcohol	204		335			339		462			
		206	205	332	334	129	372	356	468	464	108	
15	Iced alcohol											
16	Iced alcohol	202		217								
		297	250	224	220	−30						089
17	Iced alcohol	170		178								
		142	156	208	198	42						093
18	Iced alcohol	122		346								
		116	118	353	350	232						096
19a	Iced alcohol	106		86								
		127	116	57	71	−45						202
19b	Iced alcohol	192		157								
		212	202	—	157	−45						163
Average			175		218	42		350		628	278	
					No insulin							
9		125	125	155	155	+30	397	397	415	415	8	
10		64					369		153			
		82	73	76	46	+3	365	367	94	124	−243	
12		118										
		113	126	106	106	−20	409	409	304	304	−105	

TABLE 1—*Concluded*

NUMBER	METHOD	MUSCLE					LIVER					BLOOD SUGAR PER 100 CC.
		Before hydrolysis	Average	After hydrolysis	Average	Difference	Before hydrolysis	Average	After hydrolysis	Average	Difference	
		Part 2—Continued										
		No insulin—Continued										
												mgm.
15							204	204	178	178	−26	
16		163		245								
		166	164	185	215	51						114
17		122		172								
		116	119	199	186	65						109
18		107		358								
		97	102	306	332	230						113
Average			118		178	60		344		255	−921	

19a—Glycogen: Liver, 8.56; muscle, 1.2.

19b—Glycogen: Liver, 4.24; muscle, 0.18.

Remarks: Nos. 5, 9, 10, 12, 15–18, starved.

Experiments bearing the same number refer to observations made on the same day, and are parallel, so that the methods of extraction were strictly alike.

most marked in the cold extracts. The decrease may depend on the presence of some highly labile reducing substance.

3. In extracts made with hot alcohol of both muscles and liver, insulin caused a decided decrease in reducing power before and following hydrolysis. These differences are evident not only when the averages of all results of each group are taken but also, as a rule, when these are considered separately. This latter method of comparison can be made in the tables by taking the results standing opposite each of the numbers in the first column.

4. In extracts made with chilled alcohol insulin along with glucose injections caused an increase in the reducing substance before hydrolysis in muscle and a decrease in the liver. In one experiment (no. 11) on an animal injected with large amounts of sugar and insulin a most striking increase occurred in the reducing power of the extract of liver. In another animal, 13, similarly treated, the evidence for the formation of this substance is not so marked but nevertheless exists, especially when the results following hydrolysis are compared with those of starved animals.

There is, therefore, some evidence that insulin causes the free and combined sugar that is soluble in hot alcohol to become reduced in both liver and muscle. In extracts made by alcohol at low temperatures, however, administration of insulin along with sugar leads to a moderate increase in both these forms of sugar in muscle and in two instances to a more decided increase in the hydrolysable sugar in the liver. In those cases in which the blood sugar was determined immediately before killing the animals the muscle sugar both before and after hydrolysis was usually higher than in the blood. Although every possible care was taken to avoid irregularities in technique the duplicate results are far from satisfactory, which may be due to the fact that highly labile substances are being dealt with. This, of course, makes the results uncertain and further investigation is necessary.

II. THE RÔLE OF PHOSPHATES. *A. Phosphates in blood and urine.* Several workers have shown that variations in the blood sugar are accompanied by changes in the amount of inorganic phosphate in blood and urine (5). With the object of enlarging our information on these changes, especially with regard to their time relations, we have extended the experiments of Allan and Sokhey (5).

Methods. Investigation was carried out on rabbits, which were not starved, and on dogs. With the former the phosphates in both whole blood and plasma were estimated by Brigg's method, (12) together with blood sugar determinations, before and after the subcutaneous injection of insulin. With the latter, which were starved for 12 hours, 5 experiments were performed; in three of these hourly estimations of blood sugar and phosphates together with the urinary excretions of phosphate over each interval were carried out, following administration of fairly large doses of insulin. In two experiments the last estimation was not performed.[1]

Results. Table 2 gives the results on rabbits. Those with dogs are shown in figures 1, 2 and 3. In rabbits phosphate values in plasma and whole blood ran parallel, and in 4 out of 6 experiments (1, 2, 3 and 6) showed a distinct fall, following the injection of insulin. In two (nos. 4 and 5) a rise in phosphate was recorded. This fact may possibly be explained in the light of the results obtained on dogs, which show a recovery in blood phosphate some time before this occurs in blood sugar.

In dogs, following the injection of insulin there was a rapid fall in blood phosphate which ran almost parallel to the drop in glucose. The value remained low over a period of from 4 to 6 hours, when it rose fairly rapidly. In each instance, the rise in phosphate definitely preceded the rise in sugar, although at a later period, the curves might again cross (figs. 1, 2, 3).

[1] Urine was removed by catheter, and in order to ensure a sufficient volume at the hourly intervals, 50 cc. of water were given by stomach tube, immediately after each sample of blood was withdrawn.

Coincident with the fall in blood phosphate the urinary phosphate completely disappeared, a condition which persisted from 6 to 8 hours, when it reappeared in larger amounts than the normal. This latter fact substantiates the findings of Allan and Sokhey, that the 24-hour excretion of phosphate is augmented by 25 to 35 per cent.

TABLE 2

Blood phosphate. Rabbit

EXPERIMENT NUMBER	TIME	BLOOD SUGAR PER 100 CC.	PHOSPHATE PER 100 CC.	
			Whole blood	Plasma
		mgm.	*mgm.*	*mgm.*
1	12:10 p.m.		2.9	2.7
	12:15 p.m.	0.1 cc. insulin per kilogram (1 rabbit dose—0.07 cc.)		
	3:15 p.m.	56	2.6	2.5
2	12:10 p.m.		1.8	1.8
	12:15 p.m.	0.1 cc. insulin per kilogram (1 rabbit dose—0.07 cc.)		
	3:15 p.m.	Could not obtain blood	1.3	
3	10:45 a.m.		5.8	5.2
	11:03 a.m.	0.1 cc. insulin per kilogram (same strength)		
	11:30 a.m.	125		
	12:00 n.	93		
	12:30 p.m.	106	3.5	2.5
4	10:50 a.m.		5.0	4.3
	11:00 a.m.	0.1 cc. insulin per kilogram		
	11:30 a.m.			
	12:00 a.m.			
	12:30 a.m.		6.9	8.5
5	11:45 a.m.	132	3.0	
	12:07 a.m.	0.1 cc. insulin per kilogram		
	12:37 a.m.	91		
	2:07 a.m.	88	3.8	3.2
6	12:00 n.	132	4.3	Phosphate (inorganic)
	12:12 p.m.	0.1 cc. insulin per kilogram		
	12:42 p.m.	109		
	2:12 p.m.	114	2.6	2.5

In experiment 2 (fig. 2), the administration of glucose, while the animal was in a state of hypoglycemia, caused alleviation of the symptoms, coincident with a rise in blood sugar and a fall in blood phosphate.

The sudden appearance of phosphate in the urine in experiments 2 and 3 (figs. 2 and 3) would seem to indicate the presence of a renal threshold for this substance at a value from 25 to 35 mgm. per 100 cc.

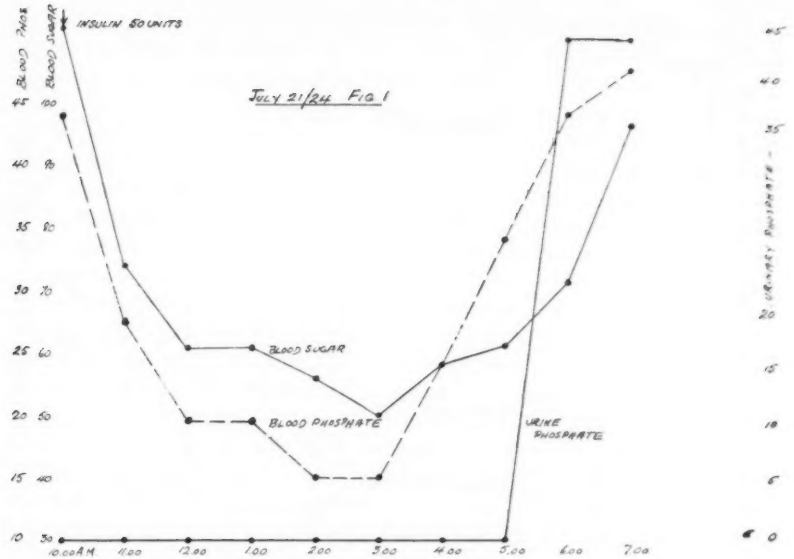
INSULIN 80 UNITS
JULY 21/24 FIG 1
BLOOD SUGAR
BLOOD PHOSPHATE
URINE PHOSPHATE
10.00 A.M.
11.00
12.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00

Fig. 1

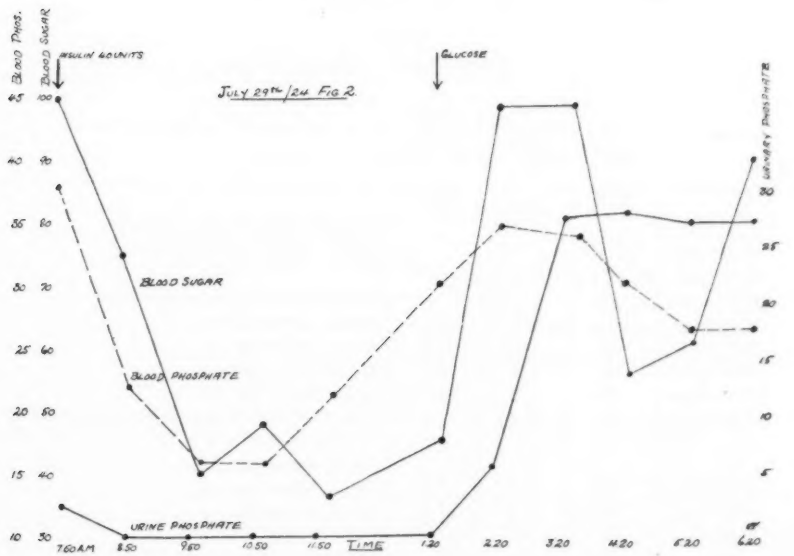
INSULIN 40 UNITS
GLUCOSE
JULY 29TH/24 FIG 2
BLOOD SUGAR
BLOOD PHOSPHATE
URINE PHOSPHATE
7.60 A.M.
8.50
9.60
10.50
11.50
TIME
1.20
2.20
3.20
4.20
6.20
6.40

Fig. 2

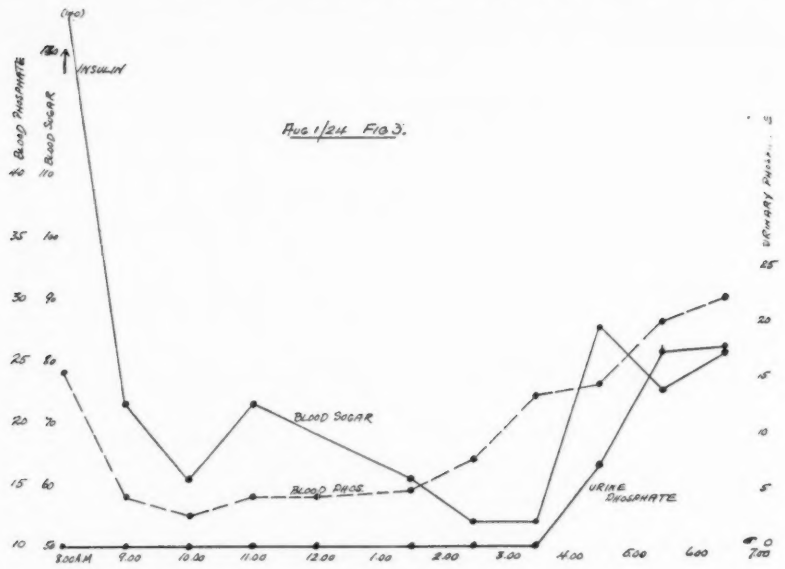

Fig. 3

The details of experiment 3 (fig. 3) are given in table 3.

TABLE 3

Dog. Weight 9 kgm. Starved 12 hours

TIME	BLOOD		URINE—PHOSPHATES PER HOUR	NOTES
	Sugar per 100 cc.	Phosphates per 100 cc.		
	mgm.	*mgm.*	*mgm.*	
7:48 a.m.				Catheterized; bladder washed
7:50 a.m.				50 cc. water by stomach tube
7:53 a.m.	100	3.8		
8:05 a.m.				40 units insulin subcutaneously
8:50 a.m.	64	2.2	3.0	50 cc. water per os
9:50 a.m.	40	1.6	0.3	50 cc. water per os
10:50 a.m.	47	1.6	0.3	50 cc. water per os
11:50 a.m.	36	2.1	0.3	50 cc. water per os
1:20 p.m.	45	3.0		Convulsions; 24 grams glucose given in 90 cc. water
				50 cc. water per os
2:20 p.m.	97	3.4	0.5	50 cc. water per os
3:20 p.m.	97	3.3	5.9	50 cc. water per os
4:20 p.m.	54	3.0	27.8	
5:20 p.m.	59	2.6	28.0	50 cc. water per os
6:20 p.m.	92	2.6	27.0	

B. Phosphates in muscle. The facts of the preceding section could be explained if the phosphate was united in some complex with the sugar entering the tissues, a complex breaking down later to release the phosphoric acid. This suggested the so-called "Lactacidogen" of Embden. Accordingly we tried to see if this substance, or rather the phosphates which are liberated in an inorganic form by incubation in a weakly alkaline solution, were increased. Embden, Laquer and their co-workers (6) believe that glucose is not utilized directly in muscle, but normally must first be changed into some other form, a form directly derivable from glycogen, though not from glucose. This intermediate substance they consider to be a hexose phosphate identical with that derived from yeast. They have found that when lactic acid is formed in muscle, phosphoric acid is produced in approximately equimolecular proportions, and regard the difference in the amounts of inorganic phosphates found before and after incubation as a measure of the amount of this precursor of lactic acid present in the muscle. The metabolism of carbohydrates in muscle normally takes this course, and it might be expected that insulin would increase the amount of "lactacidogen." On the other hand the diminished content of lactic acid found by Dudley (7) in the muscles of rabbits injected with convulsive doses of insulin would seem to indicate that the amount of lactacidogen must be less than normal. In light of this fact we would not have submitted the problem to further investigation had it not been that some authors (8) have published results indicating an increase in lactacidogen following insulin.

In undertaking this investigation it was necessary to bear in mind the fact, shown by Embden, Schmitz and Meincke (9), that strychnine convulsions decrease the amount of lactacidogen. It might be possible that convulsions due to insulin would have a similar effect. Accordingly we attempted to avoid convulsions by giving, with the insulin, sufficient glucose to prevent too great a lowering of blood sugar. It seems probable if the disappearance of phosphates from the blood be for the purpose of forming some phosphoric acid complex with glucose that the simultaneous injection of glucose would not only increase the loss of phosphates from the blood but also the amount of this compound in the muscles and this all the more so since it had been shown that the injection of glucose alone will decrease the blood phosphates (10).

Technique. Two series of experiments were performed, using for one Embden's earlier method with Buchner press-juice (9) and for the other the later method with frozen pulverized muscle (11). For the press-juice the rabbits were stunned, the muscles of the hind limbs removed, put through a sterile ice-cold meat chopper, ground in a sterile ice-cold mortar with an equal weight of sand, mixed with a tenth of the weight of kiesulguhr, pressed under 400 atmospheres in the Buchner press, and the juice

collected in a beaker surrounded by freezing mixture, precautions for sterility being taken. The analyses (in duplicate) were carried out as follows: Two samples of 5 cc. each were measured out. Sample A was treated immediately with 5 cc. of a 5 per cent solution of mercuric chloride followed by 5 cc. of 2 per cent hydrochloric acid. Sample B was incubated for 2 hours at 45°C. after the addition of 5 drops of a saturated solution of sodium bicarbonate. At the end of this time, bichloride and acid were added as in A. (In the case of experiment 1 only 3 cc. muscle juice were employed, equivalent quantities of the other reagents being added.)

TABLE 4

Phosphate in press-juice of muscle

Inorganic phosphorus; milligrams per cubic centimeter of muscle juice

	EXPERIMENT	BEFORE INCUBATION	AFTER INCUBATION	DIFFERENCE
1	Control	0.95, 0.93 } 0.94	1.39, 1.44 } 1.42	0.47
	Glucose and insulin	0.68	0.80	0.12
2	Control	1.06, 1.08 } 1.07	1.19	0.12
	Glucose and insulin	1.06, 1.06 } 1.06	1.21, 1.20 } 1.2	0.14
3	Control	1.48	1.71	0.23
	Glucose and insulin	(a) 1.31 (b) 1.08	1.70 1.20	0.39 0.12
Averages:				
	Controls	1.16	1.44	0.27
	Glucose and insulin	1.03	1.23	0.19

(Bracketed figures are duplicates.)

For the other series, the animals were stunned, the muscles of the hind limbs rapidly removed, thrown into liquid air in a large iron mortar and pulverized. Ten-gram portions of the powdered, frozen muscle were weighed out as rapidly as possible and transferred to the appropriate flasks. One set of these contained 10 cc. Locke's solution made without glucose, the other 9 cc. of this and 1 cc. saturated sodium bicarbonate; the former were immediately, the latter after two hours' incubation, precipitated with 10 cc. each of mercuric chloride and hydrochloric acid.

After standing overnight the precipitates of both series were filtered off under suction, the mercury precipitated with a stream of hydrogen sul-

TABLE 5

Frozen muscle experiments

Per cent phosphoric acid per 100 grams tissue

BEFORE INCUBATION	AVERAGE	AFTER INCUBATION	AVERAGE	LACTACIDOGEN	BLOOD SUGAR
Normals					
					per cent
0.34		0.55		0.22	
0.32	0.31	0.44	0.44	0.13	
0.30		0.44			
0.28	0.29	0.46	0.46	0.18	
0.30		0.47			
0.43	0.42	0.58	0.58	0.16	
0.40		0.58			
0.38	0.38	0.65	0.65	0.26	
0.38		0.65			
0.40	0.42	0.62	0.62	0.19	11
0.44		0.61			
Average.....	0.36		0.55	0.19	
Insulin only					
0.41		0.50		0.09	
0.34		0.47		0.13	
0.28		0.47		0.10	
0.31		0.46		0.15	
0.48	0.47	0.62	0.62	0.14	0.09
0.47		0.61			
0.34	0.33	0.52	0.53	0.19	0.07
0.33		0.53			
0.30	0.30	0.55	0.56	0.25	
0.30		0.56			
0.36	0.35	0.45	0.45	0.09	0.05
0.35		0.45			
Average.....	0.38		0.54	0.163	

TABLE 5—*Concluded*

BEFORE INCUBATION	AVERAGE	AFTER INCUBATION	AVERAGE	LACTACIDOGEN	BLOOD SUGAR
Insulin + glucose					
					per cent
0.47 0.43	0.45	0.57 0.57	0.57	0.12	0.04
0.45 0.45	0.45	0.60 0.57	0.56	0.11	0.08*
0.43 0.43	0.43	0.59 0.55	0.57	0.14	0.08
0.46 0.46	0.46	0.55 0.55	0.55	0.09	No convulsions
0.45 0.44	0.44	0.57 0.57	0.57	0.13	0.37
Average.....	0.44		0.56	0.12	

* Convulsions had occurred.

phide and after filtration the hydrogen sulphide removed by a current of air. The phosphates were then estimated by the Briggs modification of the Bell-Doisy method (12). In using the powdered muscle it was difficult to obtain very great accuracy; duplicates did not always check closely, and this for two reasons. The sample when weighed was very cold and greater or smaller amounts of water vapor condensed on it; and in the second place, the mixture obtained by precipitation of the proteins often contained lumps of material and was not very homogenous, so that it was possible that the concentration of phosphates in the fluid draining off differed more or less from that occluded in the muscle masses. This last we tried to avoid by grinding the material as thoroughly as possible in a mortar before filtration. In order to reduce as far as possible the considerable sources of error that inevitably enter into observations of this type we have, in each experiment, used, besides the injected animals, one or more normal ones as controls.

In the three experiments in which press juice was used (table 4) one, injections of insulin and sugar caused a decided reduction in both inorganic and lactacidogen phosphoric acid but in the others there was no significant change.

The experiments in which liquid air was used (table 5) were more numerous, there being six control rabbits, eight in which insulin was injected in dosage short of causing convulsions and five in which large doses of insulin

were given along with glucose. Insulin alone caused no decided change in either the inorganic or the lactacidogen phosphoric acid, although there was a slight decrease in the latter. In the animals injected with large doses of insulin and glucose there was a decided increase in inorganic phosphates accompanied by a decrease in lactacidogen. Inasmuch as the duplicates in this series of observations were fairly close as a rule, it is probable that these results are dependable.

Taking into account the irregularities among the results of different experiments, we conclude that if insulin has any effect on the distribution of phosphorus, it is very slight. There is certainly no increase in lactacidogen.

SUMMARY

1. In both muscle and liver the free and combined sugar that is soluble in hot alcohol becomes reduced after insulin. Alcohol at the freezing point of water extracts more sugar, both free and combined than hot alcohol, and in this case insulin with glucose causes a slight increase. Irregularities in the results suggest that highly labile substances are being dealt with, and the results are not conclusive.

2. It is shown that following the injection of insulin, although the drop in blood phosphate is practically coincident with that of blood sugar, the phosphate commences to rise before the sugar does, and attains its normal value first.

3. The "lactacidogen" in frozen muscle extracts is, if anything, decreased after the action of insulin and this is more marked if convulsions and marked hypoglycemia be prevented by the simultaneous injection of glucose. Decreases were found also in Buchner press extracts. Individual variations were such that it was concluded that of the factors influencing its amount, insulin is not an important one.

We wish to thank Doctor Markowitz and Mr. Orr for valuable assistance in carrying out certain of the experiments of this investigation.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

X. Uric Acid Following Total Removal of the Liver

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Received for publication March 19, 1925

In previous studies (15), (16) on the physiology of the liver, we have reported that a marked increase in the uric acid content of the blood and urine of dogs always occurs following complete removal of the liver. The magnitude of this increase of uric acid proves that the liver is directly connected with the metabolism of uric acid. The increase may be due either to an increase in the production of uric acid, or to a decrease in the destruction of uric acid formed at its normal rate, or perhaps a combination of both factors. It would seem that an evaluation of these two factors might contribute something to our knowledge of the physiology of the liver, and also of the metabolism of purines.

In this investigation we devoted our attention to the destruction of uric acid in the hepatectomized dog because this factor is predominant in the normal animal, and obviously must be evaluated before any complete quantitative studies can be made on the production of uric acid either in the normal or hepatectomized animals. The results obtained are so definite that conclusions regarding the activity of the liver in the destruction of uric acid seem justified. Incidentally we have given some conception of the quantity of endogenous uric acid formed in the dog. The numerous problems as to the origin of uric acid and the products of its destruction are beyond the scope of this paper.

Previous work on uric acid in relation to the liver. In reviewing the literature on uric acid we have limited ourselves to those studies from which inferences may be made relative to the part played by the liver in the metabolism of uric acid. For a more general review, the reader is referred to the works of Folin, (5), (6), Rose, Jones and Benedict (2).

Minkowski found a marked decrease in the uric acid content of the urine of geese following hepatectomy. Administration of urea to these animals did not increase uric acid excretion, as it does in normal birds, which clearly demonstrates that the power of synthesis of uric acid from

urea is lost when the liver is removed. Von Mach, after repeating these experiments, pointed out that the uric acid did not entirely disappear from the urine of hepatectomized birds owing to the two sources of the acid in these animals, namely, throughout the body, from metabolic processes; and in the liver, from synthesis from urea which takes place only in this organ.

That the liver of mammals also plays some part in the metabolism of uric acid was demonstrated by Pawlow (10) and his pupils in studies of the urine of dogs following the production of Eck's fistula. They found that such animals excreted a greater amount of uric acid, which they attributed to a failure of the liver to oxidize uric acid. Abderhalden, London and Schittenhelm found that this increase in the excretion of uric acid was compensated for by a corresponding decrease in the excretion of allantoin.

Perroncito removed the liver only from dogs with Eck's fistula, so that portal stasis did not result. The animals excreted urine containing such high concentrations of uric acid that crystals precipitated on cooling. Besides the large amount of uric acid excreted, there was a marked increase in the uric acid content of the blood. Mann and Magath (15), (16) have reported this same finding following hepatectomy in the dog. More recently Perroncito removed the liver from dogs and simultaneously ligated the renal arteries and veins. The animals survived from one to four hours after operation, during which time there was a definite and marked increase in the uric acid content of the blood. Control animals with ligation of the renal arteries and veins as well as ligation of the common and cystic ducts showed no increase in the uric acid content of the blood. He concluded that the accumulation of uric acid in the blood of hepatectomized animals was due to lack of destruction of uric acid.

Folin, Berglund and Derick recently published a very extensive study of the behavior of uric acid within the body. In their studies on the dog, they attempted to determine the site of destruction of uric acid. They demonstrated that uric acid injected intravenously in the dog was destroyed very rapidly, 70 per cent being destroyed the first ten minutes after injection. Excretion of the uric acid by the kidneys is a minor factor in destruction, because only traces of the injected uric acid are found in the urine and the same rapid disappearance of uric acid from the blood takes place even if the kidneys are removed. In the normal dog the renal tissue seems to be the only one capable of absorbing uric acid injected into the blood stream, since little can be detected in any of the others even if the uric acid content of the blood is greatly increased. These authors therefore concluded that this rapid destruction of uric acid in the dog took place in the circulating blood. Blood from the

femoral artery and vein contained the same amount of uric acid after injection, and no loss of uric acid could be demonstrated in the blood passing through the hind leg of the dog.

In attempting to show that the liver was not the site of uric acid destruction in the dog, uric acid was injected into a dog with Eck's fistula; its disappearance from the blood was rapid in this animal also. Further experiments demonstrated that uric acid injected into the portal vein could pass through the liver, since more uric acid was found in the kidneys than in the liver. It was also noted that the uric acid in the heart blood was greatly increased and it was shown that uric acid could be absorbed from the intestine with sufficient rapidity to cause an increase in the uric acid content of the peripheral blood.

METHODS. Since the method of removing the liver has been fully described (14), only a brief description will be given here. The operation is performed in three stages, under ether anesthesia with aseptic technic.

The first stage consists in a reverse Eck fistula; that is, lateral anastomosis of the portal vein and the vena cava, and ligation of the latter on the cephalic side of the stoma. At first a considerable portion of the blood from the posterior portion of the body passes through the liver, but since the capillaries of the liver offer more resistance to the flow of blood than is necessary for the development of collaterals through the azygos and internal mammary veins, most of the blood soon passes by way of the latter channels.

The second stage consists in the ligation of the portal vein at its entrance into the liver. This causes all the blood from the viscera and hind legs to return by way of the collateral vessels, the azygos and the internal mammary veins.

The third stage consists in removing the entire liver. The liver is removed intact with the portion of the vena cava which it surrounds, so that no trace of liver tissue remains.

Recovery from the anesthesia and the immediate effects of the operation is rapid. Within an hour the animal is, to all appearances, normal; it walks around, responds to call, and exhibits the usual interest in its surroundings.

With the administration of glucose in proper amounts, these animals may easily be kept for as long as twenty-four hours in what appears to be a normal condition. Intravenous injections of 0.5 gram of glucose for each kilogram of body weight every two hours have been very satisfactory. Any concentration of glucose solution between 50 and 10 per cent may be used, depending on the amount of fluid desired.

Two general types of experiments were conducted. In one series, following removal of the liver from a large number of dogs the accumulation of uric acid in the blood was noted, and the amount in the urine

determined at intervals corresponding usually to the periods between the taking of blood samples. Other animals were also nephrectomized at the time of hepatectomy and the uric acid content of the blood noted. Because we wished to obtain results for a period longer than a few hours, glucose was administered intravenously to most of these animals. In a few instances it was given by stomach tube, rectally, or by jejunostomy, and in some cases by combinations of these methods. Fifty per cent glucose was given as a routine, except in cases in which more fluid was desirable for promoting diuresis.

In another series of hepatectomized animals, uric acid was injected intravenously and its content in the blood and urine determined similarly to that in the hepatectomized animals to which no uric acid had been given. In a small number of this group the distribution of uric acid to the muscles was studied by removal under local anesthesia, of portions of muscle from an undisturbed site at varying intervals after injection. Control animals for both series were similarly treated except that various other surgical procedures were substituted for hepatectomy.

Specimens of blood were obtained with a dry syringe from the jugular vein and mixed with powdered oxalate in dry tubes. Specimens of urine were obtained from metabolism cages, or by catheterization and washing the bladder, and from the bladder at necropsy. If the urine was desired at definite intervals, the animal was catheterized at stated times.

Uric acid determinations were made in the blood and urine by the method of Folin and Wu, and checked occasionally by the method of Benedict (3). Similar determinations on excised muscle were made by the method described by Folin (7). Uric acid solutions for intravenous injection were prepared with the aid of lithium carbonate so that a solution, representing 2 per cent of uric acid, was obtained.

Results. The increase in the uric acid content of the blood following hepatectomy is very striking; a definite rise is always obtained within a few minutes and the content remains above normal as long as the animal survives. The course following this initial rise depends on the rate of excretion of uric acid, and in our experience has been approximately inverse to the amount excreted in the urine. With a volume of urine comparable to normal the uric acid of the blood rises from a trace to 1.5 to 2 mgm. for each 100 cc. of blood within two hours. After this time the level remains fairly constant, between 2 and 3 mgm. of uric acid for each 100 cc. of blood, as long as a normal amount of urine is being passed. If active diuresis is maintained these figures tend to be slightly lower, and if the animal becomes anuric a marked increase is noted. In the nephrectomized hepatectomized animals the increase in the uric acid content of the blood begins immediately after operation and increases gradually at a fairly uniform rate as long as the animal survives. This increase was almost

constant for the different dogs of this series, the amount rising from a trace at the time of operation to 7 to 9 mgm. for each 100 cc. of blood twelve hours after operation. The increase during the first hour appeared to be more rapid than later, but after the first hour, the increase was proportionate to the length of time after hepatectomy (fig. 1).

The accumulation of uric acid in the urine of these dogs following hepatectomy was even more striking than that in the blood. Unless the volume of urine passed is considerably above normal limits, urate crystals form almost immediately after the urine is voided, and after it has cooled to room temperature often about half of the volume is a heavy flocculent precipitate of urates. The concentration of urates in the urine is often found to be as high as 600 mgm. of uric acid in 100 cc. of urine, although a number of animals which excreted large amounts of fluid (200

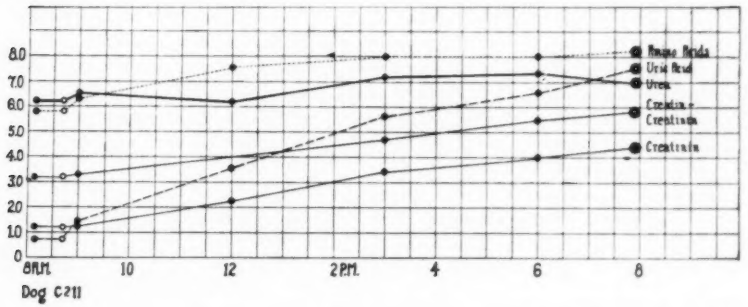

Fig. 1. Curve showing the progressive increase of uric acid in the blood of a hepatectomized dog with bilateral nephrectomy. *O*, liver and kidneys were removed. — animal died. Uric acid of blood in milligrams for each 100 cc.

cc. each hour) excreted uric acid in a much lower concentration, 20 to 30 mgm. for each 100 cc. of urine, at the same time that the uric acid content of the blood was increasing. In general, however, it may be said that the total amount of uric acid excreted depends on the volume of urine passed (table 1).

The total amount of uric acid excreted in the urine following hepatectomy is enormous. In all of our animals which had a free flow of urine for more than twelve hours, the total uric acid excretion was at the rate of 1000 to 1500 mgm. in twenty-four hours, with an hourly excretion of approximately 40 to 50 mgm. of uric acid. The daily excretion of uric acid for each kilogram of body weight was from 75 to 100 mgm., and even with this, there is an increase in the uric acid content of the blood (fig. 2).

In studying the fate of intravenously injected uric acid, we encountered

some difficulty in obtaining exact quantitative comparisons because of the variation in the rate of excretion of uric acid, which appeared to be even more marked than in the uninjected hepatectomized animal. When large amounts of uric acid were injected intravenously, the flow of urine was frequently much reduced and a few animals became entirely anuric. When smaller amounts of uric acid were injected, the flow of urine was not altered materially and large amounts of uric acid were excreted, but the amount injected was too small in comparison to the amount of uric acid excreted by an uninjected hepatectomized animal to allow exact comparisons. However, by comparing the excretion of uric acid in injected and uninjected hepatectomized animals which had comparable amounts of uric acid in the blood at the end of the period studied, large

TABLE 1

Excretion of uric acid in the urine following hepatectomy

DOG	WEIGHT, KGM.	TIME AFTER OPERATION, HOURS	INCREASE OF URIC ACID IN BLOOD, MGM.	URIC ACID INJECTED, MGM	URINE		AVERAGE EXCRETION OF URIC ACID, MGM.		CALCULATED EXCRETION OF URIC ACID, MGM.	REMARKS
					Cc.	Uric acid, mgm.	Each hour	For each kgm. body weight each hour		
G168	11.5	15	0.75 to 3.82	0	350	562	37.5	3.25		
F865	10.8	13	0.60 to 3.80	854	300	1,125			1,321*	Recovery of 85 per cent of uric acid injected

* Based on data of dog G168.

amounts of the injected uric acid were found to be excreted. Thus many of our animals appeared to have excreted from 75 to 100 per cent of the uric acid injected after removal of the liver (fig. 3).

The results obtained by observing the disappearance of injected uric acid from the blood are more satisfactory. Forty milligrams of uric acid for each kilogram of body weight were injected intravenously and the uric acid content of the blood determined five, fifteen, thirty, sixty, ninety and one hundred twenty minutes afterward. In the normal animal an increase of 2 to 3 grams of uric acid for each 100 cc. of blood is obtained at the end of five minutes after injection, ten minutes later this has almost all disappeared, and thirty minutes after injection the uric acid content of the blood is normal again. Only a trace of uric acid appears in the urine over the two-hour period.

In dogs ready for hepatectomy, that is, after recovery from operations for reverse Eck fistula and ligation of the portal vein, the blood supply to the liver is considerably altered, although the gross and microscopic appearance of the liver may be little changed. Injection of 40 mgm. of uric acid for each kilogram of body weight produces a greater rise in the uric acid of the blood and it persists longer than after smaller injections. Five minutes after injection, the amount has increased 3 to 5 mgm. for each 100 cc. of blood; ten minutes later this has decreased 1 or 2 mgm., and later, there is a more gradual decrease, so that by the end of ninety to one hundred twenty minutes the amount is normal again. Only about 2 to 10 per cent of the uric acid injected has appeared in the urine.

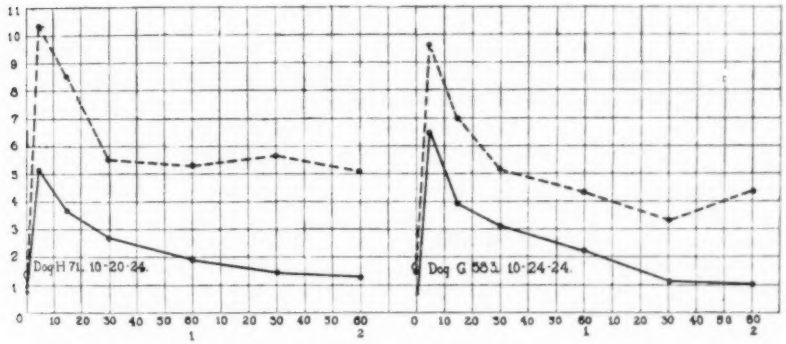

Fig. 2. Curves showing the disappearance of injected uric acid from the blood of dogs before and after hepatectomy. Ordinate, milligram uric acid for each 100 cc. of blood. Abscissa, time in minutes up to two hours after the injection of 40 mgm. uric acid for each kilogram of body weight at *O*. Before removal of the liver, solid line; after removal of liver, broken line. After hepatectomy a considerable amount of the uric acid was recovered in the urine.

After hepatectomy the injection of 40 mgm. of uric acid for each kilogram of body weight produces a much greater rise in the uric acid content of the blood. Five minutes after injection, an increase of 8 to 10 mgm. of uric acid for each 100 cc. of blood is obtained; ten minutes later this has decreased 2 or 3 mgm. for each 100 cc. of blood, and thirty minutes after injection, an increase of from 3 to 5 mgm. remains. Then the level of uric acid may remain constant for a few hours, but usually there is a gradual decrease until two hours after injection when there is an increase for each 100 cc. of blood from 1 to 3 mgm. of uric acid more than would be expected in an uninjected hepatectomized animal. Usually from 30 to 70 per cent of the injected uric acid is excreted in the urine within two hours if the flow of urine has been maintained, and the amount

of uric acid in the blood has been less in the animals excreting the greater amounts of uric acid.

In the nephrectomized hepatectomized dog, the injection of 40 mgm. of uric acid for each kilogram of body weight gives rise to even greater

TABLE 2

Experiment 474-24

PROCEDURE	TIME	URIC ACID, MGM. FOR EACH 100 CC. BLOOD	URIC ACID, MGM. FOR EACH 100 GRAMS TISSUE
Blood from jugular vein	8:30 a.m.	0.75	
Liver removed	9:00 a.m.		
Kidneys removed	9:05 a.m.		
Muscle specimen, right rectus femoris	9:15 a.m.		Trace
Blood from jugular vein	10:49 a.m.	2.90	
Injected 40 mgm. uric acid for each kilogram body weight	10:50 a.m.		
Blood from jugular vein	10:55 a.m.	11.90	
Blood from jugular vein	11:05 a.m.	8.30	
Muscle specimen, left sartorius	11:19 a.m.		7.60
Blood from jugular vein	11:20 a.m.	7.85	
Muscle specimen, left rectus femoris	11:23 a.m.		7.05
Blood from jugular vein	11:50 a.m.	7.60	
Blood from jugular vein	12:20 p.m.	7.55	
Blood from jugular vein	12:50 p.m.	7.55	
Blood from jugular vein	1:45 p.m.	8.20	
Injected 40 mgm. uric acid for each kilogram body weight	1:46 p.m.		
Blood from jugular vein	1:51 p.m.	17.70	
Muscle specimen, left vastus lateralis	1:55 p.m.		10.78
Blood from jugular vein	2:01 p.m.	16.40	
Blood from jugular vein	2:16 p.m.	15.00	
Blood from jugular vein	2:46 p.m.	14.50	
Muscle specimen, right gluteus maximus	3:10 p.m.		11.5
Blood from jugular vein	3:16 p.m.	14.00	
Blood from jugular vein	3:46 p.m.	15.40	
Blood from jugular vein	4:00 p.m.	15.90	
Injected 200 mgm. uric acid for each kilogram body weight	4:05 p.m.		
Blood from jugular vein	4:45 p.m.	41.60	
Muscle specimen, left gluteus maximus	4:45 p.m.		27.90

increases in the uric acid content of the blood. Five minutes after injection, the uric acid of the blood has increased from 10 to 12 mgm. for each 100 cc.; ten minutes later this has decreased 2 to 4 mgm., and thirty minutes later the uric acid has reached a level of 4 to 5 mgm. greater than that obtained in each 100 cc. of blood in the uninjected nephrec-

tomized hepatectomized animal at similar periods after operation. After this there is little variation in the uric acid of the blood except a gradual rise of about the same magnitude as that found in the uninjected animal. Thus the uric acid of the blood remains from 4 to 5 mgm. higher than the uric acid of the blood of an uninjected animal for at least several hours after injection. It should be mentioned here that the uric acid content of the muscles of these animals has also increased within thirty minutes after injection of uric acid, so that at this time and later the muscle contains uric acid in amounts comparable to that of the blood (fig. 4).

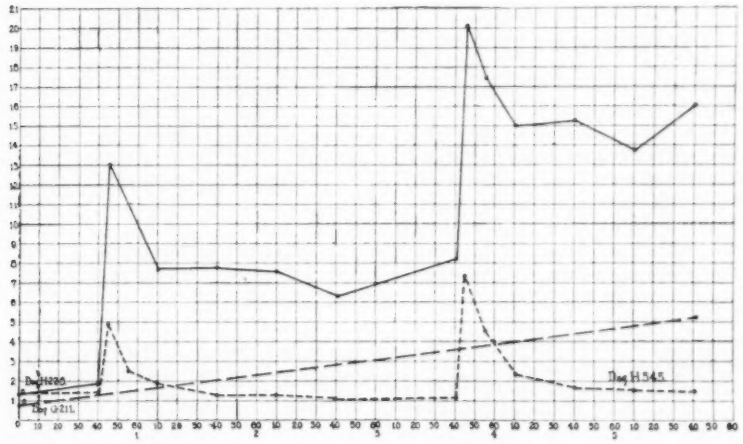

Fig. 3. Curves showing the lack of destruction of injected uric acid in the hepatectomized nephrectomized dog, H228, compared with the rapid destruction of uric acid in the nephrectomized dog, H545, and also the accumulation of uric acid in the blood of an uninjected hepatectomized nephrectomized dog, G211. Ordinate, milligram uric acid for each 100 cc. of blood. Abscissa, time in minutes. Forty minutes after operation, intravenous injection of 40 mgm. uric acid for each kilogram of body weight in dogs H228 and H545. Three hours and forty minutes after operation same injection was repeated.

After we have demonstrated that the injected uric acid in the hepatectomized dog enters the tissues in amounts similar to that of the uric acid of the circulating blood within thirty minutes after injecting 40 mgm. of uric acid for each kilogram of body weight, we can account for all the uric acid as long as the animal survives. Six hours after injecting 40 mgm. of uric acid for each kilogram of body weight, the uric acid content of the blood and tissues is higher by between 5 and 6 mgm. for each 100 grams of tissue than a similar treated uninjected hepatectomized animal with both kidneys removed. Assuming that about 75 per cent of the weight

of the animal is soft tissue, the calculated increase in the uric acid content of the blood and tissues should be 5.3 mgm. for each 100 grams of

TABLE 3

Experiment 1049-24

PROCEDURE	TIME	URIC ACID, MGM. FOR EACH 100 CC. BLOOD
October 9, 1924		
Blood withdrawn from jugular vein	8:45 a.m.	0.98
Bilateral nephrectomy performed	9:10 a.m.	
Blood withdrawn from jugular vein, animal recovered from anesthesia	10:07 a.m.	1.01
Injection completed, 40 mgm. uric acid, 0.5 gm. glucose each kilogram	10:08 a.m.	
Blood withdrawn from jugular vein	10:13 a.m.	2.72
Blood withdrawn from jugular vein	10:23 a.m.	1.45
Blood withdrawn from jugular vein	10:38 a.m.	1.12
Blood withdrawn from jugular vein	11:08 a.m.	0.95
Blood withdrawn from jugular vein	11:38 a.m.	0.90
Blood withdrawn from jugular vein	12:08 p.m.	1.16
Blood withdrawn from jugular vein	2:20 p.m.	1.28
Injection completed, 40 mgm. uric acid, 0.5 gm. glucose for each kilogram	2:21 p.m.	
Blood withdrawn from jugular vein	2:20 p.m.	3.78
Blood withdrawn from jugular vein	2:36 p.m.	1.54
Blood withdrawn from jugular vein	2:51 p.m.	1.41
Blood withdrawn from jugular vein	3:21 p.m.	1.16
Blood withdrawn from jugular vein	3:51 p.m.	0.99
Blood withdrawn from jugular vein	4:21 p.m.	1.05
October 10, 1924		
Blood withdrawn from jugular vein	9:59 a.m.	1.16
Injection completed, 40 mgm. uric acid, 0.5 gm. glucose for each kilogram	10:00 a.m.	
Blood withdrawn from jugular vein	10:05 a.m.	4.33
Blood withdrawn from jugular vein	10:15 a.m.	1.78
Blood withdrawn from jugular vein. Injected 0.25 gm. glucose for each kilogram	10:30 a.m.	1.44
Blood withdrawn from jugular vein. Injected 0.25 gm. glucose for each kilogram	11:00 a.m.	1.13
Blood withdrawn from jugular vein. Injected 0.25 gm. glucose for each kilogram	11:30 a.m.	1.16
Blood withdrawn from jugular vein	12:00 m.	1.00
Blood withdrawn from jugular vein	2:00 p.m.	1.15

soft tissue. This is the actual finding several hours after injecting the acid, so that little if any could have been destroyed during that time.

In another series of experiments the entire gastro-intestinal tract was removed with the liver and kidneys. In these animals the accumulation of uric acid in the blood has been of the same magnitude as that in the

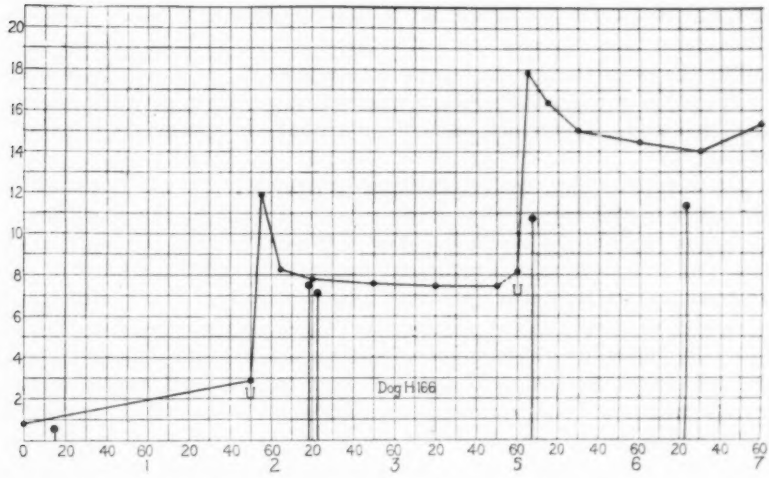

Fig. 4. Curve showing lack of destruction of uric acid in the hepatectomized nephrectomized dog. *O*, liver and kidneys removed. *U*, injected 40 mgm. uric acid for each kilogram of body weight. ⊙, uric acid content of muscle samples. Note that approximately all of the uric acid injected remains in the blood and tissues of the animal throughout the course of the experiment.

TABLE 4

Experiment 1051-23

TIME	BLOOD NUMBER	URIC ACID, MGM. FOR EACH 100 CC. BLOOD	URINE NUMBER	URINE VOLUME, CC.	URIC ACID IN TOTAL VOLUME OF URINE, MGM.
8:45 a.m.	1	0.75			
9:30 a.m.	Bladder emptied		0	0	
12:30 p.m.	2	2.43	1	70	133.7
3:30 p.m.	3	2.65	2	90	146.8
6:30 p.m.	4	2.68	3	80	123.2.
9:30 p.m.	5	2.50	4	110	122.0
12:30 a.m.	6	3.82	5	20	36.4
3:30 a.m.	7	4.70	6	0	0

hepatectomized animal with its kidneys removed. These animals have remained in good condition for a long time, eighteen hours, and there is no evidence that the absence of the gastro-intestinal tract has led to any

greater accumulation of uric acid than the absence of the liver and kidneys alone.

After studying numerous cases we decided that the rate of accumulation of uric acid and also the behavior of injected uric acid in the hepatectomized dog are not influenced by the injection of glucose. Animals living from four to seven hours after hepatectomy without glucose have shown the same changes with respect to uric acid. Most of the animals of this series were given glucose in order to prolong their life and to insure their remaining apparently normal throughout the course of the experiment.

Discussion. The results of these experiments are not complicated by many of the factors that have influenced most of the experiments involving exclusion of the liver. The liver was removed in one piece so that not a trace remained in the animal and the other tissues were not affected noticeably, either grossly or microscopically. The loss of blood at operation was slight and the operation rapid. Usually, within an hour after the beginning of the ether anesthesia the liver has been removed, the anesthetic discontinued, and the dog has appeared normal again, having recovered from the anesthesia. In all cases the animal continued to appear normal throughout the course of the experiment, sometimes more than twenty-four hours. We consider a dog normal when it walks around, responds to call, wags its tail and exhibits the usual interest in other dogs and its surroundings. In brief, the animal appears similar to one that has recovered from ether anesthesia and any surgical procedure.

The lack of destruction of uric acid following removal of the liver indicates that this organ is responsible for its destruction in the normal animal. Uric acid injected intravenously is almost quantitatively recovered in the urine of the hepatectomized dog. Complete quantitative studies in this respect are extremely difficult because of the variations in the rate of excretion of uric acid before and after its injection as the kidneys were often affected thereby. However, since it was found that the rate of accumulation of uric acid in anuric hepatectomized animals was uniform, a comparison may be made between the amount of uric acid excreted by animals with similar concentration of uric acid in the blood. In this manner almost quantitative recovery of uric acid injected intravenously is obtained in the urine. In animals with the liver and both kidneys removed the entire amount of uric acid injected can be determined in the blood and tissues several hours after injection. The injected uric acid enters the tissues rather slowly so that its concentration in the blood is greater than that in the tissues for some time after injection, but the amount in the muscles gradually approaches that of the circulating blood. It should also be noted that the rate of accumulation of uric acid in our double nephrectomized hepatectomized animals was

comparable to the accumulation calculated on the assumption that the amount of uric acid usually excreted by a hepatectomized animal is being retained and none is being destroyed. Usually the hepatectomized animal excretes 40 to 50 mgm. of uric acid for each kilogram of body weight in twelve hours, with an increase of the uric acid in its blood to between 2 and 3 mgm. for each 100 cc. The uric acid of the blood and muscles of a hepatectomized animal with both kidneys removed has increased to between 8 and 9 mgm. for each 100 cc. of blood, or 100 grams of tissue. This amounts to an accumulation of from 40 to 50 mgm. of uric acid for each kilogram of body weight, if it is assumed that about 75 per cent of the animal's weight is soft tissue.

The large amount of uric acid excreted by the hepatectomized dog is extremely interesting. The excretion of from 80 to 100 mgm. of uric acid for each kilogram of body weight in twenty-four hours might imply an increased production, as well as lack of destruction of uric acid. Our only findings tend to oppose such a view, since there is no increased nitrogen excretion (urea formation ceases) (5) and the creatin-creatinin excretion is not altered after removal of the liver. The excretion of uric acid nitrogen in the hepatectomized dogs is of the same magnitude as that accepted (1), (20) as the excretion of allantoin nitrogen in the normal dog. Oddly enough, it is more than twice the amount of the uric acid plus allantoin excretion of the Dalmatian hound (2), (7) which excretes from three to four times as much uric acid as man. The excretion of uric acid by the hepatectomized dog is ten times greater than that of man.

SUMMARY

Large amounts of uric acid appear in the urine of dogs following hepatectomy which also gives rise to an increase in the uric acid content of the blood. Uric acid injected intravenously into animals following hepatectomy may be almost quantitatively recovered in the urine. Uric acid injected into animals with the liver and both kidneys removed is slowly absorbed by the muscles so that it does not all remain in the circulating blood. Determination of the uric acid content of the blood and muscles several hours after injection shows that the total amount is retained in the body and that but little of it could have been destroyed. The uric acid accumulated in the body of hepatectomized nephrectomized dogs is of the same magnitude as that excreted by the kidneys of hepatectomized animals. The uric acid excreted by the hepatectomized animal is of the same magnitude as the allantoin excreted by the normal dog. The destruction of uric acid in the normal dog depends on the presence of the liver as this process ceases as soon as the liver is removed.

PROTOCOLS. We have selected only a few representative experiments from our series of more than 200 hepatectomized animals in which the changes in uric acid were noted.

Experiment 589-20. This is one of our earlier experiments showing an increase in the uric acid content of the blood following removal of the liver, a finding which has been observed in every animal studied in our series of hepatectomized dogs.

Dog D918 was a black and brown male, adult mongrel hound in good condition, weighing 13.2 kgm. August 30, 1920, a reverse Eck fistula was performed. September 17, the portal vein was ligated. The animal remained in good condition, and October 12, weighed 14.5 kgm. On this day the following experiment was performed:

8:30 a.m., blood sample 1 was taken from the jugular vein.
9:00 a.m., anesthesia was started.
9:20 a.m., the liver was removed; it weighed 286 grams.
9:40 a.m., the operation was completed.
10:30 a.m., the animal appeared normal.
12:00 m., the animal showed signs of muscular weakness which were promptly dispelled by intravenous injection of 50 cc. of 20 per cent glucose solution.
1:30 p.m., the animal voided 85 cc. of urine.
4:30 p.m., 50 cc. of 10 per cent glucose solution were injected intravenously.
4:35 p.m., the animal was given 40 cc. of a 20 per cent glucose solution by mouth.
4:45 p.m., the animal voided 100 cc. of urine.
6:30 p.m., the animal was given 40 cc. of 20 per cent glucose solution by mouth.
8:00 p.m., the animal voided 65 cc. of urine.
8:30 p.m., the animal was given 40 cc. of a 20 per cent glucose solution by mouth.
10:30 p.m., the animal was given 40 cc. of a 20 per cent glucose solution by mouth.
12:00 m., blood sample 2 was taken from the jugular vein.
12:30 a.m., the animal was given 40 cc. of a 20 per cent glucose solution by mouth.
1:45 a.m., 100 cc. of a 10 per cent glucose solution were injected intravenously.

The animal was left in the kennel and was found dead at 7:00 a.m., apparently a short time after death. Blood sample 3 was withdrawn from the heart. Necropsy 527-20 was performed immediately. The site of the operation was in excellent condition. There was no hemorrhage into the peritoneal cavity. The bladder contained 187 cc. of urine.

BLOOD NUMBER	TIME AFTER OPERATION		URIC ACID, MGM. FOR EACH 100 CC.
	hours	*minutes*	
1	0	0	Trace
2	14	40	1.0
3	21	40	3.1

Experiment 474-24. This experiment shows the lack of destruction of uric acid injected intravenously into a hepatectomized nephrectomized dog. The entire amount of uric acid injected plus the amount of uric acid normally found in the hepatectomized animal may be accounted for as the amount of uric acid found in the blood and tissues of the animal. The amount of uric acid destroyed is too small to be detected.

Dog H166 was a black, brindle and white adult male bull terrier in good condition weighing 12.3 kgm. May 7, 1924, a reverse Eck fistula was performed. October

15 the portal vein was ligated. The animal remained in good condition and December 11 weighed 12.2 kgm. On this day the following experiment was performed.

8:30 a.m., blood sample 1 was removed from the jugular vein.

8:50 a.m., the anesthesia was started.

9:00 a.m., the liver was removed, it weighed 310 mm.

9:05 a.m., both of the kidneys were removed.

9:15 a.m., muscle specimen 1, a portion of the right rectus femoris, was removed.

9:25 a.m., the operation was completed and the anesthetic discontinued. The animal weighed 11.3 kgm. post-operatively.

9:50 a.m., 22.6 cc. of 50 per cent glucose solution were injected intravenously.

10:49 a.m., the animal had recovered from the anesthesia and appeared to be normal. Blood sample 2 was withdrawn from the jugular vein.

10:50 a.m., the injection into the jugular vein of 22.6 cc. of a solution containing 452 mgm. of uric acid and 2.82 grams glucose (40 mgm. uric acid, 0.25 gram glucose for each kilogram of body weight) was completed.

10:55 a.m., blood sample 3 was taken from the jugular vein.

11:05 a.m., blood sample 4 was taken from the jugular vein.

11:19 a.m., muscle specimen 2, a portion of the left sartorius, was removed under local anesthesia.

11:20 a.m., blood sample 5 was taken from the jugular vein.

11:23 a.m., muscle specimen 3, a portion of the left rectus femoris, was removed under local anesthesia.

11:50 a.m., blood sample 6 was taken from the jugular vein; 5.7 cc. of 50 per cent glucose solution were injected into the jugular vein.

12:20 p.m., blood sample 7 was taken from the jugular vein; 5.7 cc. of 50 per cent glucose solution were injected into the jugular vein.

12:50 p.m., blood sample 8 was taken from the jugular vein; 5.7 cc. of 50 per cent glucose solution were injected into the jugular vein.

1:45 p.m., blood sample 9 was taken from the jugular vein.

1:46 p.m., the injection into the jugular vein of 22.6 cc. of a solution containing 452 mgm. of uric acid and 2.82 grams glucose (40 mgm. uric acid; 0.25 gram glucose for each kilogram of body weight) was completed.

1:51 p.m., blood sample 10 was taken from the jugular vein.

1:55 p.m., muscle specimen 4, a portion of the left vastus lateralis, was removed under local anesthesia.

2:01 p.m., blood sample 11 was taken from the jugular vein.

2:16 p.m., blood sample 12 was taken from the jugular vein.

2:46 p.m., blood sample 13 was taken from the jugular vein.

3:10 p.m., muscle specimen 5, a portion of the right gluteus maximus, was removed under local anesthesia.

3:16 p.m., blood sample 14 was taken from the jugular vein and 5.7 cc. of 50 per cent glucose solution injected.

3:46 p.m., blood sample 15 was taken from the jugular vein and 5.7 cc. of 50 per cent glucose solution injected.

4:00 p.m., blood sample 16 was taken from the jugular vein.

4:05 p.m., the injection into the jugular vein of 113 cc. of a solution containing 2.26 grams of uric acid and 2.82 grams of glucose (200 mgm. uric acid, 0.25 gram glucose for each kilogram of body weight) was completed.

4:45 p.m., blood sample 17 was taken from the jugular vein. Muscle specimen 6, a portion of the left gluteus maximus, was removed under local anesthesia. Table 2 contains the data obtained in this experiment.

The animal was killed and necropsy (948-24) performed immediately. The liver and kidneys were found to have been cleanly removed. No signs of hemorrhage were found. Nothing was found that would, in any way, invalidate the results of this experiment.

Experiment 1049-24. This experiment shows the rapid destruction of uric acid in the dog with both kidneys removed.

Dog H549 was a brown and white female collie, in good condition, and weighing 16.9 kgm. The protocols of this experiment are condensed in table 3.

At 2:00 p.m., September 10, 1924, the animal was killed. Necropsy (755-24) was performed immediately. Both kidneys were removed completely and no signs of hemorrhage appeared. Nothing was found that would invalidate the results of this experiment.

Experiment 315-24. This experiment shows an approximately quantitative recovery of uric acid injected intravenously into a hepatectomized dog when a flow of urine is maintained. Absolute quantitative data of this kind are difficult to obtain since injections of large amounts of uric acid often produce marked renal disturbances.

Dog F865 was a black and white adult male mongrel in good condition, weighing 11.9 kgm. April 4, 1924, a reverse Eck fistula was performed. July 3, the portal vein was ligated. September 9, the following experiment was performed:

8:30 a.m., blood sample 1 was withdrawn from the jugular vein.

8:45 a.m., anesthesia was started.

8:55 a.m., the liver was removed; it weighed 272 grams.

9:10 a.m., the operation was completed and the anesthetic discontinued. The animal weighed 10.8 kgm. post-operatively.

9:40 a.m., the animal appeared to be normal. The bladder was emptied by catheter. Blood sample 2 was withdrawn from the jugular vein, and 10.8 cc. of a 1 per cent uric acid solution in 50 per cent glucose were injected.

10:40 a.m., blood sample 3 was taken from the jugular vein and the injection repeated.

11:40 a.m., blood sample 4 was taken from the jugular vein and the injection repeated.

12:40 p.m., blood sample 5 was taken from the jugular vein and the injection repeated.

2:00 p.m., the injection was repeated.

3:00 p.m., blood sample 6 was taken from the jugular vein and the injection repeated.

4:20 p.m., blood sample 7 was taken from the jugular vein and the injection repeated.

5:30 p.m., the injection was repeated.

7:00 p.m., 10.8 cc. of 50 per cent glucose solution were injected.

8:15 p.m., the glucose injection was repeated.

9:15 p.m., the glucose injection was again repeated.

10:55 p.m., blood sample 8 was taken from the jugular vein.

11:55 p.m., blood sample 9 was taken from the jugular vein.

12:10 a.m., blood sample 10 was taken from the heart, and the animal killed.

Necropsy (706-24) was performed immediately. The liver was completely removed and no signs of hemorrhage appeared. The kidneys were congested and swollen. The bladder contained 300 cc. of clear urine. The total amount of urine

excreted, 300 cc., contained 1125 mgm. of uric acid. The total amount of uric acid injected was 864 mgm. Uric acid determinations in the blood were:

BLOOD NUMBER	URIC ACID, MGM. FOR EACH 100 CC. BLOOD
1	0.60
2	1.90
3	2.50
4	3.10
5	3.50
6	3.65
7	3.65
8	3.20
9	3.80
10	4.20

Experiment 1051-23. This experiment is typical of many showing the great increase in the uric acid content of the blood and urine following hepatectomy. The amount of uric acid excreted in the urine is about the same as the average, although with urinary retention there may often be less uric acid in the urine but more in the blood.

Dog G168 was a black and white adult female mongrel in good condition, weighing 11.6 kgm. December 20, 1923, a reverse Eck fistula was performed. On January 24, 1924, the collateral circulation was not sufficiently developed, and only partial occlusion of the portal vein was made. July 27 the portal vein was completely ligated. September 2 the following experiment was performed. The animal weighed 12.4 kgm.

8:45 a.m., blood sample 1 was withdrawn from the jugular vein.

8:55 a.m., anesthesia was started.

9:20 a.m., operation was completed and anesthesia discontinued. The animal weighed 11.5 kgm. after operation and the liver weighed 331 grams.

9:30 a.m., the animal was catheterized and the bladder emptied. One-fourth gram of glucose for each kilogram of body weight (23 cc. of a 12.5 per cent glucose solution) was injected into the jugular vein.

10:30 a.m., the glucose injection was repeated.

11:30 a.m., the glucose injection was again repeated.

12:30 p.m., urine specimen 1 was catheterized, blood sample 2 was withdrawn, and the intravenous injection of glucose was repeated.

1:30 p.m., the glucose injection was repeated.

2:30 p.m., the glucose injection was again repeated.

3:30 p.m., urine specimen 2 was catheterized, blood sample 3 was withdrawn, and the intravenous injection of glucose repeated.

4:30 p.m., 0.5 gram of glucose for each kilogram of body weight (23 cc. of 25 per cent glucose solution) was injected.

6:00 p.m., 0.25 gram of glucose for each kilogram of body weight (23 cc. of 12.5 per cent glucose solution) was injected.

6:30 p.m., urine specimen 3 was catheterized, blood sample 4 withdrawn, and 23 cc. of 25 per cent glucose solution injected.

7:55 p.m., 23 cc. of 12.5 per cent glucose solution were injected.

8:40 p.m., 23 cc. of 25 per cent glucose solution were injected.

9:30 p.m., urine specimen 4 was catheterized, blood sample 5 withdrawn, and 23 cc., 12.5 per cent glucose solution injected.

10:30 p.m., 23 cc. of 25 per cent glucose solution were injected.

11:40 p.m., 23 cc. of 12.5 per cent glucose solution were injected.

12:30 a.m., urine specimen 5 was catheterized, blood sample 6 withdrawn, and 23 cc. of 25 per cent glucose solution injected.

2:00 a.m., the injection was repeated.

3:30 a.m., blood sample 7 was withdrawn, and the animal killed. Table 4 contains the pertinent data of this experiment. Necropsy (694-24) was performed at once. The bladder was empty. Nothing was found that would invalidate the results of this experiment.

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MINERAL METABOLISM STUDIES WITH DAIRY CATTLE

Mineral Equilibrium After Prolonged Lactation[1]

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Received for publication March 12, 1925

In a previous article (1) we published results pertaining to mineral balances in three Holstein cows during the early lactation period. The work herein reported deals with results obtained after the same three animals had been maintained under successful lactation for ten months and were still farrow. This departure from the ordinary dairy practice was purposely directed in order to study the equilibrium of the inorganic radicles after a prolonged period of lactation. At the time of this experiment the animals were still producing about 20 pounds of milk daily.

The quality of feed during the intervening period was the same as during the mineral balance observation. The average daily consumption of silage of these three cows varied from 30 to 35 pounds, animals 216 and 218 consumed 15 to 18 pounds of red clover hay and animal 222 from 10 to 15 pounds. The usual practice of reducing the grain with decline in milk production was followed. Ten days preceding the quantitative record of the mineral balance, animals 216, 218 and 222 were daily consuming 16.5, 17.8 and 14.3 pounds of red clover hay respectively. Four days prior to our quantitative collection of excreta and during the entire period of seven weeks, each animal was daily given 15 pounds of clover hay, 30 pounds of oats and vetch silage, and 1 pound of grain mixture (corn 58, mill run 32, oil meal 10) to 3.5 pounds of milk produced. During the 4th, 5th, 6th and 7th weeks, the rations of animals 216 and 222 were supplemented with 150 grams of bone meal daily, animal 218 was given 20 pounds of green kale during the first 18 days of the same period. The method of collecting the samples and the analytical procedure used were the same as outlined in the previous publication.

Discussion of results. The basal ration and other factors involved favored a storage of phosphorus in all cases, and a storage of calcium by two of the animals. Inasmuch as the hay consumption of animal 218 was lowered due to our arbitrarily fixing the amount at 15 pounds for all

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the animals, a positive calcium balance would most likely result if she were allowed free access to hay. A decided increase in calcium and phosphorus storage resulted from supplementing the ration with bone meal. Apparently any excessive drain on the body supply of calcium and phosphorus may be compensated for by increasing the plane of calcium and phosphorus intake through bone meal feeding. The question of a positive balance and quantity of latter is largely dependent on the degree of depletion of the body supply as shown by Hart and co-workers (2). Analysis of the blood plasma for calcium and inorganic phosphate indicates a higher concentration of these elements as a result of bone-meal feeding. The calcium concentration of the plasma from the animals on the basal ration is slightly below the average, but this is brought up to the average after the supplements are introduced into the ration. Kale apparently caused a marked increase in plasma calcium, but the phosphate concentration in the plasma remained approximately the same. This is in agreement with the increased storage of calcium by the organism. The inorganic phosphorus was determined by the method of Briggs (4) and the calcium according to Halverson (5).

The data given in the mineral-balance tables show a positive and favorable balance for all the inorganic elements except potassium. The potassium balance for animals 216 and 222 is negative throughout the entire seven weeks. The negative potassium balance is comparatively greater in animal 218 during the first three weeks, but changes to a positive balance after supplementing with kale. This is just the reverse of what was observed during early lactation when kale was introduced.

As observed during early lactation, green kale again caused and maintained an increased milk yield after a ten-month period of continued milk production. In the early lactation period green kale caused the phosphorus absorption from the intestines to increase from 28 to 38 per cent and a negative phosphorus balance was changed to one of equilibrium. This time the positive phosphorus balance was decreased, but the absorption of calcium was greatly increased and calcium storage occurred. These differences in mineral retention of the organism caused by the same variety of feed is further demonstrated in a study of the acid-base equilibrium.

Acid-base equilibrium. In addition to considering the mineral elements from the standpoint of depletion and storage, it is also of physiological interest to recognize the relationship in acid and base economy that exists from these mineral metabolism studies. For units of comparison cubic centimeters of normal solutions are used. Thus the balance of sodium, potassium, magnesium and calcium which form the "base" are calculated in terms of normal solutions of alkali and also the balance of sulfur, phosphorus and chlorine calculated in terms of normal acid. Potassium sodium and chlorine are monovalent; calcium, magnesium and sul-

fate are divalent. Phosphorus here is calculated as divalent, although in the body it is 1.8 (3). The undetermined amounts of phosphorus and sulfur excreted which are not oxidized to acid radicles prevent this calculation from being absolutely accurate. In expressing values of acid-base metabolism the term "base balance" is used. A positive base balance results from base retention or acid excretion in excess of intake; a negative base balance results from base excretion in excess of intake or acid retention. From the mineral balances reported in tables 1, 2 and 3 and calculating as stated above, the "base balances" are given in table 5.

A negative base balance results with all three animals on the basal ration at this time in their production period due to the loss of bases from the body and acid retention. When the ration is supplemented with bone meal there is base and acid retention, but the latter being in excess leaves a negative base balance, although less than it was during the first period. The base retention obtained is due almost entirely to the calcium storage resulting from the bone meal supplementing the ration. If the increased quantities of calcium and phosphorus stored due to the supplement are calculated in terms of bone meal from the percentages of each element found on analysis, it is found that 48.0 grams of bone meal were required to supply the 13.4 grams of calcium and approximately 29.0 grams to supply the increased phosphorus retention of 4.5 grams in animal 216. Similar results are obtained with animal 222. Since the amounts of calcium and phosphorus excreted in the urine and secreted in the milk were no greater, these results show that the excess of the acid radicle was eliminated by the intestine and whether it be through selective ion absorption by the epithelium or absorption of the salt and excretion of phosphorus into the intestine, the latter has functioned in regulating the acid base relations in the body. A condition of acid retention and loss of base on the basal ration was, to a certain degree, counterbalanced after bone meal feeding by excretion of more acid by way of the intestine and storage of base by the animal. This is also substantiated in the ratio of acid to base in the feces. During the basal-ration period the ratio of base to acid in the feces (table 5) is greater than during the period where the feed was supplemented with bone meal and also where kale was added as a supplement. Regardless of the nature of the supplement the animals at this period responded to this addition to their diet in such a manner that there was a shift in the acid-base equilibrium in favor of a positive base balance. Calculations from data received in our former work reveal that the shift in acid base equilibrium was just the opposite when the same supplements were added, that is, the change was from a positive to a negative base balance. This is in harmony with the difference in the calcium and phosphorus balance due to the kale supplement observed in animal 218, phosphorus retention being favored in the earlier run and calcium in the present investigation.

TABLE 1

Average daily mineral balance animal 216

PERIOD	AVERAGE DAILY MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		Feed	Feed	Feed	Feed	Feed	Feed	Feed	Feed
		Milk	Milk	Milk	Milk	Milk	Milk	Milk	Milk
		Urine	Urine	Urine	Urine	Urine	Urine	Urine	Urine
		Feces	Feces	Feces	Feces	Feces	Feces	Feces	Feces
		Balance	Balance	Balance	Balance	Balance	Balance	Balance	Balance
	kilos	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*
November 23 to December 14, 21 days basal ration	9.25	214.8	16.1	34.0	34.6	90.3	33.2	157.5	18.0
		47.3	3.0	8.7	6.3	9.8	1.0	13.4	5.4
		70.6	2.0	0.4	6.3	0.3	5.4	127.0	5.2
		92.7	9.4	22.1	16.8	79.1	30.0	22.9	8.0
		+4.2	+1.7	+2.8	+5.2	+1.1	−3.2	−5.8	−0.6
December 14 to January 11, 28 days basal ration plus 150 grams bone meal daily	9.00	216.0	16.6	57.1	35.1	132.3	33.9	157.5	18.3
		46.2	3.0	8.8	6.6	9.6	0.9	12.6	5.2
		66.0	2.3	0.4	5.8	0.2	5.2	135.0	7.2
		102.1	9.9	40.6	17.0	108.0	29.3	16.2	5.3
		+1.7	+1.4	+7.3	+5.7	+14.5	−1.5	−6.3	+0.6

TABLE 2

Average daily mineral balance animal 218

PERIOD	AVERAGE DAILY MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance	Feed Milk Urine Feces Balance
	kilos	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*
November 23 to December 14, 21 days, basal ration	10.40	225.7 52.6 71.8 99.9 +1.4	17.0 3.4 2.7 8.8 +2.1	36.6 8.8 0.4 24.4 +3.0	38.7 8.0 11.7 13.2 +5.8	90.6 11.6 0.2 82.0 −3.2	34.4 1.2 8.3 26.7 −1.8	159.4 14.9 139.9 16.8 −12.2	20.6 5.3 16.6 3.5 −4.8
December 14 to January 11, 28 days, basal ration plus 9.07 kilos green kale daily (18 days, December 14 to January 1)	11.92 (December 14 January 4 10.33 (January 4 to 11)	251.8 59.8 73.0 98.7 +20.3	22.9 3.9 5.9 11.0 +2.1	40.0 10.0 0.4 28.3 +1.3	47.6 9.1 19.9 12.8 +5.8	107.1 13.0 0.3 88.8 +5.0	37.1 1.3 7.0 27.2 +0.7	179.0 16.6 138.2 16.4 +7.8	21.4 5.7 13.1 3.2 −0.6

TABLE 3

Average daily mineral balance, animal 222

PERIOD	AVERAGE DAILY MILK YIELD	NITROGEN	SULFUR	PHOSPHORUS	CHLORINE	CALCIUM	MAGNESIUM	POTASSIUM	SODIUM
		Feed	Feed	Feed	Feed	Feed	Feed	Feed	Feed
		Milk	Milk	Milk	Milk	Milk	Milk	Milk	Milk
		Urine	Urine	Urine	Urine	Urine	Urine	Urine	Urine
		Feces	Feces	Feces	Feces	Feces	Feces	Feces	Feces
		Balance	Balance	Balance	Balance	Balance	Balance	Balance	Balance
	kilos	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*	*gram*
November 23 to December 14, 21 days basal ration	9.84	222.8	16.8	35.9	37.6	90.5	34.1	158.8	20.0
		45.4	2.9	7.9	7.0	10.3	1.2	13.2	6.8
		77.8	2.3	0.5	13.8	0.7	7.5	141.0	12.1
		88.0	9.1	24.0	11.3	79.1	25.5	11.1	1.5
		+11.6	+2.5	+3.5	+5.5	+0.4	−0.1	−6.5	−0.4
December 14 to January 11, 28 days basal ration plus 150 grams bone meal daily	9.05	220.0	17.1	58.8	37.5	131.6	34.5	157.5	20.1
		41.6	2.7	7.2	6.4	9.3	1.1	11.4	6.1
		75.5	2.9	0.5	13.3	0.2	7.3	142.6	9.3
		85.7	9.6	44.5	13.9	112.1	25.1	8.5	2.6
		+19.2	+1.9	+6.6	+3.9	+10.0	+1.0	−5.0	+2.1

The positive potassium balance in animal 218 falls in line with the general tendency to store base. It is difficult to see how the persistent negative potassium balance in animals 216 and 222 interferes with the immediate physiological requirements because of the large quantities excreted from the circulation by way of the kidney. The presence of carbonates and an alkaline reaction of the urine preclude the possibility of there being a

TABLE 4

Calcium and inorganic phosphorus content of blood plasma

Cow	MGM. Ca IN 100 CC. BLOOD PLASMA				MGM. INORGANIC P IN 100 CC. BLOOD PLASMA	
	Basal ration		Basal ration plus bone meal or kale		Basal ration	Basal ration plus bone meal or kale
216	8.40	8.85	9.60	9.60	4.00	6.25
218	7.80	7.35	10.03	9.90	3.90	4.25
222	8.08	8.10	8.85	9.36	3.75	6.13

TABLE 5

Daily base—balance expressed in cubic centimeters of n/10 solution per kilogram of weight

Column 5 gives ratio of acid to base in feces calculated on basis of normal solution.

ANIMAL	RATION	n/10 SOLUTION	REMARKS	RATIO ACID:BASE
		cc.		
216	Basal ration	15.0 negative	Base lost, acid retention	1:2.95
	Basal ration plus bone meal	4.5 negative	Acid and base retention	1:2.28
218	Basal ration	23.0 negative	Base lost, acid retention	1:2.75
	Basal ration plus kale	1.8 positive	Acid and base retention	1:2.52
222	Basal ration	12.6 negative	Base lost, acid retention	1:2.63
	Basal ration plus bone meal	20.0 negative	Acid and base retention	1:2.06

special demand for potassium to neutralize the acid radicles. Evidently some of the potassium excreted through the kidney would be available to make up any serious deficiency of potassium in the organism.

SUMMARY

1. Continuing our former work, a quantitative determination of the nitrogen, sulfur, phosphorus, chlorine, calcium, magnesium, potassium

and sodium balance was made with the three cows after a ten-month lactation period. The animals were farrow and were producing about 20 pounds of milk daily.

2. The basal ration consisted of 15.0 pounds of clover hay, 30 pounds of silage and one pound of grain (corn 58, millrun 32, oil meal 10) to 3.5 pounds of milk. In a second period this ration was daily supplemented with 150 grams of bone meal for two cows and the third cow received daily 20 pounds of kale as a supplement.

3. At this period the animals stored calcium and phosphorus on the basal ration.

4. Supplementing with bone meal caused increased storage of calcium and phosphorus, a marked increase in inorganic phosphate content and a slight increase in the calcium content of the blood plasma.

5. Kale again caused an increase in milk production, calcium in the plasma and calcium storage. Differing from the former period, the phosphorus storage was not increased.

6. Introducing bone meal or kale into the ration caused the acid-base equilibrium to shift from a negative base toward a positive base balance. Absorption from the intestine or secretion into the intestine appears to be the regulatory mechanism in controlling the above balance.

7. The conditions of this experiment favor a storage or equilibrium of all the inorganic elements except potassium in two of the animals. It seems safe to infer that the large quantities of potassium excreted in an alkaline urine preclude the possibility of there being a serious deficiency of potassium in the organism.

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THE ASYNCHRONISM OF RIGHT AND LEFT VENTRICULAR CONTRACTIONS AND THE INDEPENDENT VARIATIONS IN THEIR DURATION

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Received for publication February 21, 1925

INTRODUCTION. While it is now definitely established (1), (2) that the beginning and end of systole occur sooner in the right auricle than in the left, it is still the current teaching that the two ventricles start and end their contractions, as well as the ejection of blood, simultaneously. Indeed many analyses of cardiodynamic events necessarily predicate simultaneous activity of the two hearts; and many conceptions of the behavior of the heart under varying conditions are based on the fundamental assumption of a synchronous action of the two ventricles. Thus, a myocardiograph is applied to the heart and the resultant graph is taken as an expression of the activity of either ventricle; a manometer is inserted into one ventricular chamber and the experimenter feels assured that the beginning and end of the separate cardiac phases thus established holds for either ventricle. When a cardiometer is placed around the ventricles, the resulting volume curves are only analyzable because we have the supreme conviction that the two sides of the heart begin and end their ejection quite concordantly, etc. When, however, we examine the existing experimental evidence upon which such a far-reaching generalization is based, we find the more reliable data apparently not in support of such a conception. Thus, Lewis and Rothschild (3) found the spread of the excitation so ordered that the major portion of the surface of the right ventricle is excited somewhat earlier than the left. The question naturally arises whether, under such modes of excitation, the two ventricles could begin their contractions synchronously.

Differences in the end of right and left ventricular contraction might be expected even if the onset were synchronous for it is well known that the chief factors found by Wiggers and Katz (2), (4), (5) to determine the duration of the systolic phases (viz., arterial resistance and initial tension and length) are quantitatively different in the right and left hearts and are usually altered more on one side than the other when conditions change.

Direct attempts to study the synchronism of ventricular contractions were studied by Stassen (6) and Fauconnier (7). They found that normally the right ventricle began to contract before the left. The cumbersome myocardiographs which they attached to either ventricle were too inefficient and too inaccurate, however, to make their results more than suggestive. Eppinger and Rothberger (8) found an asynchronism of about 0.01 of a second at the onset of ejection on recording the aortic and pulmonary pulses by means of solenoid recorders. They attributed this asynchronism to differences in the isometric phases and not to asynchronous contraction, however. A more direct suspicion that differences occur in the onset and cessation of right and left ventricular contraction was obtained by Wiggers (9) during the course of observations involving the recording of left and right ventricular pressure curves; but inasmuch as these records were not sufficiently checked as regards parallax, final conclusions could not be drawn.

METHODS. In this research the time relations of homonymous events in the right and left hearts were investigated by means of optically recorded pressure curves which make possible precise determinations of the separate phases of their respective cycles. This report represents observations obtained on 24 dogs under morphine and chloretone anesthesia. The usual technical procedures employed in exposing the heart were followed.

The new type of optical manometer recently described by Wiggers and Baker (10) was used as a recording instrument. These were used in pairs, one to record the precise sequence of events in the systemic circuit, the other corresponding events in the lesser circuit. They were inserted either into the right and left ventricular chambers or into the aorta and pulmonary artery just distal to the semilunar valves. The technique was practically the same as that described elsewhere by Wiggers (5) except in two essential details: 1, the pulmonary manometer was inserted through the pulmonary conus after the manner described by Schäfer (11); and 2, the double slit arc lamp which was developed especially for this research and reported by Katz and Baker (12) was substituted for the ordinary projector. With this illuminating arrangement *parallax was entirely avoided* by placing the side tube of the two manometers in the same horizontal plane.

A control record was taken after all manometers had been properly aligned. Records were also obtained under a variety of conditions, i.e., at various stages during and after stimulation of the peripheral end of the right and left vagus nerve, during infusion of saline into the right auricle, during various stages of asphyxia, while the lungs were inflated and deflated, during and after compression of the thoracic aorta just above the diaphragm and likewise during and after manual compression

of the pulmonary artery just before its bifurcation. In each experiment, a control record was obtained for comparison. The technique used in producing these changes has been described by Wiggers (5). Undesired heart rate changes were avoided by cutting the vagi nerves. Respiration was temporarily interrupted while records were being taken, to avoid the complications produced by its action.

The records so obtained were analyzed for significant changes in diastolic blood pressure or initial intraventricular tension, for alterations in the height of the arterial and intraventricular curves and for changes in contour. No effort was made to calibrate the manometers and the results are qualitative and relative rather than quantitative and absolute. In order to obtain these measurements base lines were recorded as lettered in figure 1. The most important part of the analysis, however, consisted in determining accurately:

1. Time differences, if any, between the onset of the pressure rise in right and left ventricles and between the ends of right- and left-sided systoles.

2. Time differences, if any, between the beginnings and ends of right and left ventricular ejections.

3. The durations of total systole, systolic ejection, previous diastole and the entire cycle in both ventricles.

For this purpose the criteria described by Wiggers (5) were employed. These are shown as vertical lines in figure 1. Only records with clearly defined points were measured and all the measurements were made by the author.

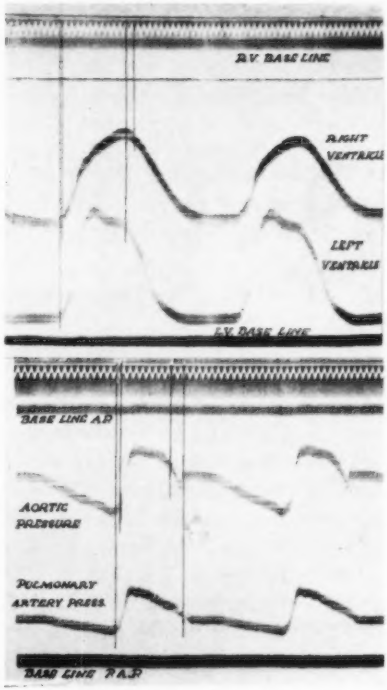

Fig. 1. Segments of records showing relations of right and left intraventricular pressure curves (upper set) and pulmonary and aortic pressure curves (lower set). Vertical lines indicate method of calculating degree of asynchronism in onset and end of systole or ejection. Note in upper curves that left ventricular systole precedes the right very slightly as to onset but that the right ventricular systole ends 0.029 later then the left; also in the upper curve right ventricular ejection precedes left by 0.01 second and continues 0.04 of a second after the left. Time 0.02 of a second. (One-half original size.)

In all 4137 simultaneous right and left ventricular cycles were analyzed and the data recorded in a preliminary fashion on specially constructed forms. About one-third of these cycles deal with ejection and the remaining two-thirds are concerned with total systole. The data cover some 150 pages and obviously cannot be reproduced here. From this mass of figures twenty summary tables showing the range of the variations were made and in many instances when the variations were very marked from beat to beat, plots were made on coördinate paper. The tables and plots so constructed were studied and analyzed and form the immediate source of the material presented in this report. Even these tables and plots are too extensive for publication, consequently word descriptions must necessarily be substituted, or a few typical experiments selected to represent the group.

THE ASYNCHRONISM IN THE ACTIVITY OF THE TWO VENTRICLES UNDER NORMAL EXPERIMENTAL CONDITIONS. The first task undertaken was to determine the degree of asynchronism under what may be called normal experimental conditions, that is, shortly after the apparatus was properly aligned and while the vagi were still intact. Typical records are shown in figure 1.

Thirty-nine sets of observations were made and tabulated both in detailed and condensed form. Two of the latter tables are reproduced in tables 1 and 2. A study of such tables showed that at least under experimental conditions *neither the beginnings nor ends of systole or ejection are synchronous events in the two ventricles.* In fact, synchronism is the exception and asynchronism the rule. Furthermore, there is apparently no consistent relation in this asynchronism. Not only does the amount of asynchronism vary from animal to animal, but also from time to time in the same animal (e.g., expts. 4, 14 and 17, tables 1 and 2): slight variations occurring even from beat to beat.

The following details may be summarized:

In seven out of thirteen animals ejection began in the right side before the left, in three dogs blood began to leave the left ventricle before the right, in two, ejection began reasonably simultaneously, while in the remaining animal (expt. 10—table 1) sometimes the right and at other times the left ventricle was the first to eject its blood. The degree of asynchronism in most instances is not negligible. A few figures taken from table 1 may be given as an indication of the differences obtained: Precedence of right-sided ejection ranged from 0.000 to 0.036 of a second in nine experiments. Precedence of left-sided ejection ranged from 0.008 to 0.025 in three experiments.

Similar variations are found when the beginning of contraction is analyzed. In three dogs the right ventricle began to contract first, in six the reverse was the case. In two instances, observations 10 minutes

TABLE 1

Asynchronism of right and left ventricular ejection

EXPERIMENT	ASYNCHRONISM OF ONSET		ASYNCHRONISM OF END		TOTAL DURATION OF RIGHT EJECTION	TOTAL DURATION OF LEFT EJECTION	CYCLE LENGTH
	Right precedes	Left precedes	Right stops before left	Left stops before right			
	second	*second*	*second*	*second*	*second*	*second*	*second*
1	0.004–0.016	—	—	0.016–0.022	0.132–0.139	0.104–0.112	0.360
2	0.000–0.009	—	—	0.033–0.039	0.254–0.260	0.212–0.220	0.596
3	0.029–0.036	—	—	0.002–0.010	0.126–0.131	0.080–0.091	0.440
4a	0.015–0.018	—	—	0.006–0.020	0.138–0.145	0.110–0.120	0.425
4b	0.008–0.020	—	—	0.018–0.026	0.174–0.182	0.134–0.145	0.410
5	0.012–0.024	—	—	0.004–0.014	0.147–0.162	0.124–0.132	0.400
6	0.012–0.024	—	—	0.013–0.024	0.205–0.213	0.164–0.176	0.414
7	0.000–0.008	—	—	0.032–0.046	0.198–0.210	0.152–0.166	0.555
8	0.010–0.016	—		0.001–0.006	0.099–0.106	0.082–0.090	0.336
9	0.020–0.022	—	0.010–0.020	—	0.100–0.117	0.100–0.105	0.440
10	0.002–	–0.008	—	0.010–0.028	0.090–0.111	0.078–0.097	0.290
11	—	0.010–0.020	—	0.014–0.025	0.099–0.107	0.094–0.102	0.385
12	—	0.018–0.025	—	0.015–0.025	0.152–0.165	0.152–0.165	0.450
13	—	0.008–0.010	—	0.000–0.010	0.209–0.218	0.212–0.220	0.790

TABLE 2

Asynchronism of right and left ventricular systole

EXPERIMENT	ASYNCHRONISM OF ONSET		ASYNCHRONISM OF END		TOTAL DURATION OF RIGHT SYSTOLE	TOTAL DURATION OF LEFT SYSTOLE	CYCLE LENGTH
	Right precedes	Left precedes	Right stops before left	Left stops before right			
	second	*second*	*second*	*second*	*second*	*second*	*second*
14a	0.000–0.008	—	—	0.034–0.070	0.255–0.278	0.200–0.229	0.595
15	0.020–0.027	—	—	0.000–0.015	0.160–0.172	0.127–0.144	0.350
16	0.000–0.016	—	0.008–0.022	—	0.128–0.151	0.140–0.156	0.325
17a	0.000–0.010	—	0.032–0.040	—	0.205–0.226	0.238–0.250	0.475
18	0.004–0.014	—	—	—	—	—	—
14b	—	0.013–0.017	—	0.026–0.029	0.195–0.200	0.182–0.188	0.509
19	—	0.002–0.020	—	0.015–0.035	0.199–0.221	0.187–0.206	0.410
20	—	0.009–0.022	—	0.002–0.020	0.208–0.236	0.210–0.238	0.515
21	—	0.007–0.018	—	0.004–0.015	0.151–0.170	0.158–0.168	0.370
22	—	0.030–0.035	—	0.016–0.026	0.167–0.183	0.180–0.190	0.340
23	—	0.014–0.032	—	0.000–0.008	0.134–0.160	0.156–0.184	0.390
17b	—	0.004–0.012	0.015–0.028	—	0.138–0.152	0.174–0.178	0.348
24	—	0.003–0.016	—	—	—	—	—

apart (expt. 17—table 2) and 70 minutes apart (expt. 14—table 2) showed a reversal of precedence. It is interesting to note that in both of the last two animals auricular fibrillation and flutter had followed vagal stimulation or had occurred spontaneously. The range of asynchronism at the onset of contraction is the same order as the range of asynchronism at the onset of ejection. The following figures taken from table 2 indicate the ranges: Precedence in inception of right ventricular contraction ranged from 0.000 to 0.027 of a second in five experiments. Precedence in inception of left ventricular contraction ranged from 0.002 to 0.035 of a second in eight experiments.

When the results are combined one notes that there are as many animals in which the onset of systole begins earlier in the right ventricle as there are those in which the onset of systole or systolic ejection starts sooner in the left.

Obviously the asynchronism in the onset of contraction and ejection may be due *a*, to differences in the conduction times on the two sides, or *b*, to differences in the interval elapsing between excitation and contraction of a sufficient number of muscle elements to elevate the initial tension in the two ventricles. If the first interpretation is adopted we must assume that variations in impulse conduction in the ventricles not only occur in different animals but that the conduction rate may change in the same animal as well. If, on the other hand, the latter interpretation is accepted it is not improbable that differences in initial tension and length or differences in aortic resistance may in part account for this state of asynchronism.

The relations of the end of systole and of the phase of systolic discharge are also shown in the typical records of figure 1 and the range of variations is tabulated in tables 1 and 2. An examination of the tabulated experiments shows that with but three exceptions (expts. 9, 17a and 16—tables 1 and 2) the contraction of the right heart outlasts that of the left for a variable time. In the three exceptions, however, the reverse is the case. The end points in the other two animals were too indefinite to permit accurate readings. The later termination of the systole and systolic ejection of the right ventricle, in most of the cases forms a sharp contrast to the inconstant relation of the onset in this series. The range of asynchronism of the end is also much greater than the range of the onset, as is shown by the following figures, taken from table 1 and 2: Right ventricular systole or systolic ejection terminates before left by 0.008 to 0.04 of a second in three animals. Left ventricular systole terminates before right by 0.000 to 0.07 of a second in 19 animals.

The asynchronism of the end of systole is not entirely dependent on the asynchronism of the beginning but varies independently. Thus, in eleven experiments the contraction ends sooner in the left heart even

though it starts later there. In one experiment only is the reverse the case (expt. 17b—table 2), contraction starting sooner in the left ventricle but ending later. Even in the other experiments where there is a qualitative similarity, quantitative differences are often present. In the entire series there are only four experiments where the asynchronism of the beginning and end are at all comparable; in all the others there are qualitative or at least quantitative differences.

The effects of these time differences on the asynchronism of the two ventricles as well as on the relative durations of their respective contractions can be better visualized by the aid of a diagram such as is shown in

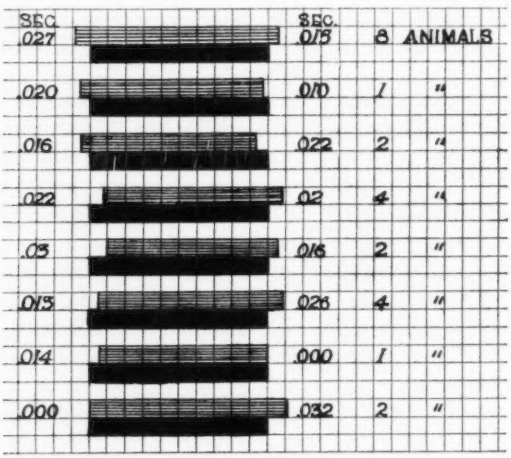

Fig. 2. Diagrammatic summary of results in twenty-four experiments, showing relative onsets, and termination of right and left ventricular systole and ejection periods. Solid blocks represent duration of left ventricular systole taken as 100. Shaded blocks indicate relative onset and termination of right ventricular systole or ejection. Figures indicate mean values of the asynchronism observed in each group of cases, the number of which is also indicated.

figure 2. In this figure the duration of total systole or of systolic ejection on the left side is taken as a constant value of 100 per cent (black blocks) and the beginning and end of right ventricular systole or ejection related to it (shaded blocks). A glance at this diagram shows that:

1. Right ventricular systole or ejection begins earlier and ends later than left ventricular systole or ejection in eight experiments.

2. Right ventricular systole or ejection begins earlier and ends earlier than left ventricular systole or ejection in three experiments but the time differences at the beginning and end are not equal.

3. Right ventricular systole or ejection begins later and ends later than left ventricular systole or ejection in ten experiments, but the time differences at the beginning and end are relatively equal in only four experiments.

4. Right ventricular systole or ejection begins approximately simultaneously with and ends later than left ventricular systole or ejection in two experiments.

These differences in the onset and termination of systole or systolic ejection necessarily produce differences in the duration of these events in two ventricles. This is also evident in figure 2. Simple calculation or measurements show that in twenty-four experiments right and left ventricular systoles are approximately equal in four cases only.

The greatest difference in the duration of right and left ventricular systole observed is 0.079 of a second but this must probably be regarded as exceptional. Frequently, the difference is about 0.040 of a second but in the majority of instances about 0.020 of a second. Returning once more to the actual data of table 2 it will be found that in three of the nine animals in which total systole was measured the right ventricular systole is longer; in four, the left systoles are longer and in the other two they are about of equal duration. The relationships of right and left ejection phases are more consistent as shown in table 1, the ejection being longest in the right ventricle, in all but two animals in which the duration of the ejection phase is approximately equal on the two sides.

The relatively greater length of the right ventricular ejection may be explained by the fact that the pressure in the pulmonary artery is decidedly less than in the aorta and that the duration of ejection bears an inverse ratio to the arterial resistance against which it works (5), (2). However, other factors no doubt account for some of the differences in the relative duration of right and left systole and ejection.

We may conclude, therefore, that although there is no constant relation in the duration of the total contraction period in the two ventricles, the *ejection phase is definitely longer in the right ventricle.* Providing the systolic discharge of the two hearts is equal, and it is logical to assume that this is the case, then the longer ejection phase of the right ventricle proves that the rate at which blood is ejected by this heart is slower than the rate in the left heart. *In other words, the two pumps not only work asynchronously but also eject blood at different velocities.*

VARIATIONS IN ASYNCHRONISM DURING HEART-RATE CHANGES—STIMULATION OF THE PERIPHERAL END OF THE VAGUS. The peripheral ends of the severed vagi were stimulated in order to determine what effect the resultant changes in heart rate had on the asynchronism of the two ventricles. The stimulation was performed under high and low arterial resistance as well as when the venous inflow was large and small. Tables

compiled from the data in fifty-five such experiments, however, showed no qualitative difference in the results, each animal having a "type reaction" under all these conditions.

It was further noted that large variations in the relative onset and end of right and left ventricular systole as well as ejection occur from beat to beat during the stimulation and after its cessation. Consequently, the results of the best experiments were plotted in detail on coördinate paper in such a way that the relative beginnings and terminations of systole or ejection could be compared at a glance, without regard for the actual values of the systoles.

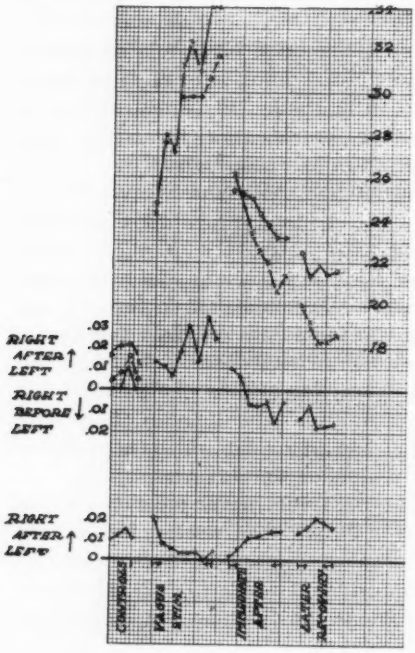

Fig. 3. Plot showing effects of stimulating peripheral vagus nerve on duration of systole (uppermost curves) on relative termination of right and left ventricular systole (middle curve) and on relative onsets of right and left ventricular systole (lower curves) in consecutive cycles. In upper curve, x represents duration of right systole; circles, left systole as interpreted in terms of ordinates on right hand side. Left ventricular systole is taken as constant, between the two horizontal lines marked o. *Beginning* of right systole is plotted in relation to the lower line; *end* of right systole to the upper line. Full description in text.

Figures 3 and 4 are examples of such plots. The asynchronism of the two ventricles is shown in the lower half of the charts. The beginnings and ends of left ventricular systole or ejection are indicated by two horizontal lines arbitrarily placed a definite distance apart. The lower line represents the onset, the upper, the termination of these events in the left ventricle. The absolute variations in the duration of systole are ignored in this way, the value of left systole being considered constant at 100 per cent regardless of its actual value. The beginning and end of the right systole or ejection are plotted as dots around these lines. When right systole begins or ends before the left, the dot is placed below the corresponding line. When it begins or ends after the left, the dot is placed above the line. The vertical distance of the dot from the line shows the amount of asynchronism. As such plots

cannot be used to compare the relative lengths of right and left ventricular systoles, the actual duration of the two systoles in these beats is indicated in the upper half of the chart, *X*, indicating right, and *O* left systole.

A study of thirty such plots of the best experiments led to the following conclusions:

In most of the experiments the right ventricle begins to contract or to eject blood relatively earlier during stimulation than before. In the experiments where the right ventricle begins to contract after the left in the

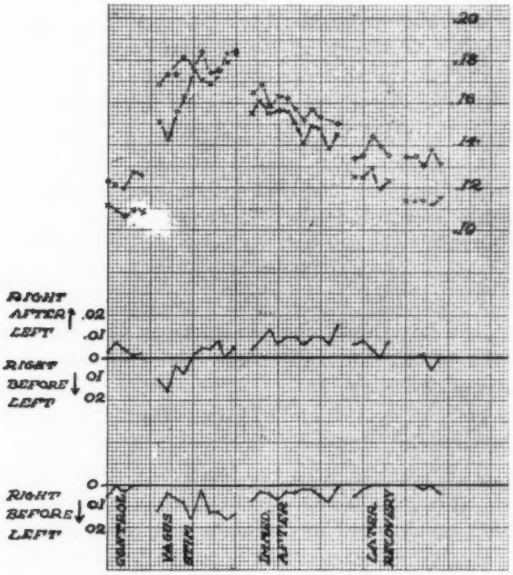

Fig. 4. Plot showing effects of stimulation of the peripheral end of the vagus nerve on the asynchronism of right and left systole and their durations, constructed similarly to figure 3. Fully discussed in text.

controls, the two ventricles either begin more nearly synchronously (fig. 3) or the right actually begins before the left during stimulation. In the experiments where the onset of right ventricular systole precedes the left in the control, the interval between the two increases during stimulation (fig. 4). Such results show that in some way vagus stimulation tends to advance the onset of right ventricular contraction in relation to the contraction of the left. Whether this is due to an earlier excitation in the right heart or a delayed excitation in the left cannot be determined from these results.

Not all of the experiments showed such regular and constant changes as outlined above. In a relatively small number the reverse effect was observed, viz., the right ventricle during stimulation tends to start contracting later in relation to the left than in the controls. When there was a left precedence at the onset, this is increased during stimulation.

In certain animals vagus stimulation produced great irregularities from beat to beat both in the onset of the two ventricular contractions and in the beginning of their ejection. Sometimes these consist of relatively large time differences similar but much greater than those shown in figure 4. In extreme instances the relative onsets of right and left ventricular systoles are actually reversed. Such an instance is shown in the segments of figure 5. As shown in the normal control of segment A, the contraction of the left ventricle precedes the right by 0.039 of a second. In the first beat after vagus stimulation, shown in segment B, the relationship is reversed, the right ventricle definitely preceding by 0.026 of a second. This is followed by a totally blocked ventricular impulse, not shown, which is followed by the beat shown in segment C. Here the ordinary relationship is restored, the left ventricle again definitely preceding the right by 0.042 of a second. This precedence slowly increases until by the eighth beat (segment D), the interval equals 0.054 second.

Such irregularities strongly support the view that the differences in the onset of right and left ventricular contractions are attributable to a modification of the conduction rate below the common A–V bundle (cf. also observations of Drury (13)).

As regards the ends of systole and ejection, stimulation of the peripheral vagus tends to delay these events more in the right ventricle than in the left. This together with the relatively earlier onset of right ventricular systole and ejection has the effect of lengthening ventricular systole and ejection much more in the right ventricle than in the left. These effects, which were characteristic in most of the experiments, are clearly shown in the graphs of figure 3.

In a few experiments, right systole terminated relatively sooner than in the control before stimulation. In most of these experiments right ventricular systole still lengthened relatively more than the left because the change in the relative onset was so marked. This is illustrated in figure 4. Only in a few instances did the systole of the right ventricle lengthen to a lesser extent than the left.

To sum up these findings we may say that in the majority of the experiments right ventricular systole and ejection begin relatively earlier during vagus stimulation than in the controls. In a larger number of cases the right ventricular systole and ejection tends to end relatively later during stimulation than in the controls and this occurs very often in spite of the opposite tendency of the onset of contraction or ejection. The net result

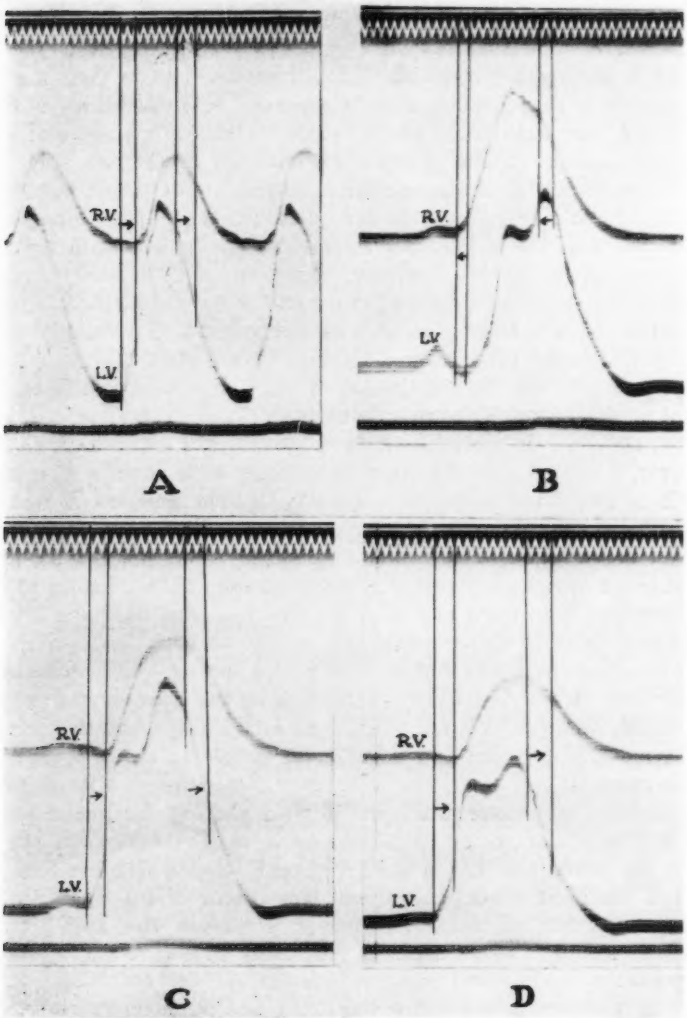

Fig. 5. Four segments of pressure curves before and during stimulation of right vagus nerve. *R.V.*, right ventricle, *L.V.*, left ventricle. Vertical lines indicate relative onsets and terminations of right and left ventricular systoles, arrows indicating precedence. *A*, normal control; *B*, *C* and *D* represent first, second and eighth beats after stimulation. Discussion in text. (One-half original size.)

is that in most cases the lengthening of systole and ejection is greater on the right side; although in a smaller number of cases the reverse effects are noted.

Why is the right ventricular systole lengthened more than the left? The answer to this question must be separated into discussions as to the cause of *a*, the earlier beginning of right ventricular systole, and *b*, the later termination of right ventricular systole, for as we have seen these contribute in varying degree to the lengthening observed. It has already been suggested that the establishment of abnormal conduction rates below the common bundle is probably responsible for differences in the onset of contractions in the two ventricles. This can hardly explain the greater prolongation of the contraction process on the right side, particularly as this often occurs in association with an earlier onset. Previous investigation by Wiggers and the writer (2) seemed to indicate that some relationship exists between the lengthening of left ventricular systole and the initial tension or volume of this ventricle on the one hand and, to a lesser extent, the diastolic aortic pressure on the other. Certain other findings, however, indicated that still other factors may be involved e.g., a poorer nutrition associated with the lessened diastolic pressure, slowed rate (perhaps enhanced by a vagal coronary vasoconstriction), etc.

Some one or other of these factors might be responsible for the longer continuance of right ventricular systole, but from the knowledge at hand it is impossible to draw any final conclusions. The following observations pertaining to this question are worthy of note, however: 1. The initial tension in both ventricles increases during vagus stimulation, particularly in the first beats. Inasmuch as the manometers were not calibrated, however, it is not possible to establish quantitative relations between initial pressure and duration of systole in the two ventricles. While this relationship deserves a far more careful study, it is my impression that the differences in the end of right and left ventricular systoles can not be accounted for by differences in inital tension. In the first place, the initial pressure in the left ventricle apparently increases more than in the right when predominant lengthening of the right ventricle occurs. Further, as vagal stimulation continues the initial tension decreases again, in some instances practically to normal (fig. 5) yet the disproportion in left and right ventricular systoles continues. 2. The diastolic pressure falls both in the aorta and pulmonary artery. The fall is more marked in the aorta, as is shown by the greater actual shift in the recorded aortic curve despite the lesser sensitivity of the aortic manometer as compared with the pulmonary. As a matter of fact, a rise in pulmonary pressure very often precedes the fall. Such changes might explain the greater prolongation of the left ventricular contraction observed in a few cases, but they cannot explain the greater prolongation on the right side usually found.

These observations indicate that some other factor than initial tension and length or arterial resistance is responsible for the greater prolongation of right ventricular systole and that this unknown factor is to be sought among those acting directly on the inherent contractile properties of the heart muscle.

After-effects. It has previously been pointed out by Wiggers and Katz (2), (4), (5) that the effects of vagus stimulation tend to persist for some beats after the cessation of stimulation and that these after-effects on different functions of the heart pass off at different times. Thus, the pacemaker is the first to recover and sometimes this recovery goes beyond normal causing a slightly faster rate. The prolonging effect on systole persists longer, however, so that we may have a stage where systole is longer and the cycle is shorter than normal.

These effects were also present in experiments from which the plots of figures 3 and 4 were made. In comparing the duration of systole in the right and left ventricles during recovery it is found quite consistently that the duration of the right ventricular contraction diminishes more rapidly than that of the left (cf. upper curves—figs. 3 and 4). These more persistent differences in the relative lengths of right and left ventricular contractions are largely due to differences in the relative termination of right and left ventricular contractions and much less, as shown in figures 3 and 4, to differences in onset from the normal.

Summarizing all of these observations then we may say that after vagus stimulation there is a tendency for ventricular systole to remain prolonged even when the heart rate has been restored to normal or is a trifle faster. This after-prolongation is relatively greater in the left ventricle and is due to a relatively longer continuation of the contraction process on this side.

INFLUENCES AFFECTING INDEPENDENT CHANGES IN RIGHT AND LEFT VENTRICULAR CONTRACTION. Having shown 1, that during normal experimental conditions, right and left ventricular systoles neither begin nor end simultaneously nor are of the same duration, and 2, that these relations are altered by changes in heart induced by simple vagus stimulation, further attempts were made to evaluate the factors responsible for independent changes in the contraction of the two ventricles.

Relative changes in durations of right and left ventricular systole may obviously be occasioned *a*, by differences in the onset of contractions, and *b*, differences in their terminations. The former, as already intimated, are most probably explained by changes in conduction rates. The latter, however, are more probably associated with direct dynamic effects on muscular contractions. Inasmuch as previous work (2), (4), (5) has clearly shown that an increase in initial tension and initial volume operates to increase the duration of the contraction process and an augmentation

of aortic resistance has the reverse effect, i.e., produces a shortening, a series of experiments was devised which it was hoped would test the effect of these influences as regards the relative termination of right and left ventricular systole.

A. The influence of venous infusion. Previous experiments from this laboratory (2), (4), (5) have shown that intravenous infusion of saline into a jugular vein increases the initial tension in both ventricles and prolongs both the ejection phase and total systole of the left ventricle. It has not been determined, however, whether the relative onset and termination of right and left ventricular contractions remain unaltered. A study of right and left ventricular systoles when the maximum effects of infusion were manifested was therefore made in twelve experiments. The results showed that with two exceptions the prolongation of systole and systolic ejection affects both ventricles, though not to an equal degree. In two experiments, the prolongation was greater on the right side; in six experiments, the left ventricle was affected more; while in two other experiments, the prolongation was equal on the two sides. In the remaining two experiments, left ventricular systole was practically unaffected while right ventricular contraction increased.

Differences in the relative onset of the two ventricular contractions were not great (exceeding 0.010 second only in one instance). In seven experiments no alteration could be calculated, in three experiments the right ventricle started its contraction slightly later than normally as regards the left, while in the other two the right ventricle started to contract relatively earlier to a slight degree. The differences in systole length were therefore almost entirely due to an asynchronous cessation of systole in the right and left ventricles. Right ventricular systole terminated relatively earlier than normally in six experiments and relatively later in four. These changes were often of considerable magnitude, frequently being as great as 0.026 of a second.

It should be noted that in most of these experiments the heart cycle was also somewhat lengthened, but that this was not the sole cause of the systolic changes is shown by four experiments where similar changes were observed although the cycle length remained unaffected.

During the course of the infusion a number of interesting variations in the behavior of the ventricles were noted. In some cases, as in that illustrated in figure 6, the lengthening of right and left ventricular ejections occurred in progressive fashion. In this figure the onset of left ventricular systole is taken as the zero line to which the other events are related. We note that before infusion, *A*, right ventricular ejection precedes the left and that the asynchronism diminishes slightly as saline is infused, *B-G*. Right ejection is so much longer than the left at the start, *A*, that it actually ends after the left, despite the precedence at

the onset. As saline is infused both ejection phases lengthen, the right to a greater extent, however, thus increasing the asynchronism at the end, *B-F*. Associated with these changes there is a slight prolongation of the cycle which is, however, insufficient to explain the lengthening of ejection.

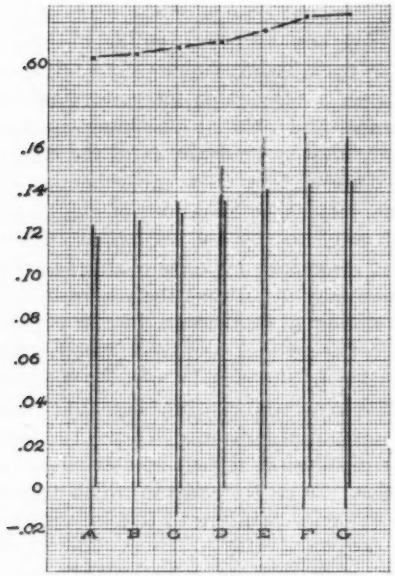

Fig. 6

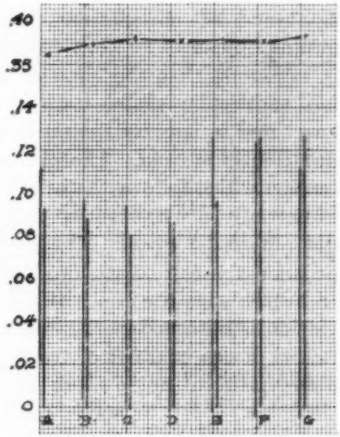

Fig. 7

Fig. 6. Plot showing the effect of infusion of saline into the right auricle on the asynchronism of the right and left ejections and on their durations. In each couple of lines, the left hand one indicates right ejection and the right hand one, left ejection. Upper curve shows the changes in cycle duration. *A*, is control; *B*, *D*, various stages during infusion. Discussed in text. Figures on ordinates in seconds.

Fig. 7. Plot showing the effect of infusion of saline into the right auricle on the asynchronism of the right and left ejections and their durations. Constructed as figure 6. In each couple of lines, the left hand one indicates right ejection and the right hand one, left ejection. To obtain the sequence of events start at the bottom and read upwards. Figures on ordinates in seconds.

In other instances, as illustrated in figure 7, the first tendency seems to be for systolic ejection to shorten and only as infusion is continued does the ejection phase lengthen to and beyond normal. These changes do not effect the two ventricles to an equal degree. The abbreviation is more marked in the left ventricle and the lengthening that follows

occurs first on this side, *D;* but is soon exceeded by the right ventricle, *E*. An abbreviation of the right ejection phase is again observed toward the end, *G*, although the left sided ejection phase is still lengthening slightly. The consequent changes in the asynchronism of the end are clearly shown and from this chart one can appreciate that the change in the asynchronism at the end is sometimes due to the change in the onset as from *A* to *B* and at other times the unequal change of the contraction phase on the two sides as *D* to *F*. All of these changes it will be observed occur with practically no variation in cycle length (upper curve, fig. 7).

The changes observed may be given a reasonable interpretation on the basis of changes in initial tension and arterial resistance in the right and left circulation. However, positive evidence as to the determining influences at work cannot be deduced from these experiments. It is apparent that the changes in the relative terminations of right and left ventricular systoles are in part produced by the variations in the onset and in part by the unequal and sometimes opposite changes in the duration of systole. The prolongation may mean that the rise of initial tension which follows the infusion is not equal on the two sides or that it is partially neutralized by the rise in the arterial pressures, which may not always be equal. The abbreviation in the systolic phases, when it occurs early, can be attributed to a rise in arterial resistance great enough to overbalance the effect of the greater initial tension. When an abbreviation is observed in the last stages of infusion, it may be attributed to failure of one or other of the ventricles. In all the cases in which a final abbreviation is noted there is other evidence pointing to this interpretation, viz., a fall in aortic pressure associated with a rise in pulmonary pressure and with changes in the contour of the ventricular curves. Alterations in the contour of both the ventricular and arterial curves sometimes accompany the abbreviation of systole in the earlier stages and indicate that some other influences than those mentioned above are responsible for the change in the duration of contraction.

B. Compression of the thoracic aorta. Since it had previously been shown by Wiggers and Katz (2), (5) that an increased arterial resistance abbreviates left ventricular systole it was hoped to establish by compression of the aorta that left ventricular systole can be abbreviated without appreciably affecting the duration of right ventricular systole. Unfortunately, as is now realized, the aorta was compressed far too centrally to produce such simple effects. In the first place, as an analysis of the records has shown, the dynamic effects on the left ventricle are often complicated by an incomplete emptying which increased the initial tension and diastolic volume—dynamic changes which have the reverse effects on duration of total systole and systolic ejection. In the second place, compression of the aorta is not without effect on the right heart

as was clearly indicated in the optical pressure curves. The effect is twofold: on the one hand it tends to cause a lessened venous return, resulting in a lower initial tension, lower pressure maximum and reduction of pulmonary pressures; on the other, it may cause a variable back-pressure effect which tends to increase not only the pulmonary arterial resistance but the initial tension in the right ventricle as well. The balance of these opposing influences determines the changes in the duration of right ventricular systole and systolic ejection. It is to be expected therefore that the temporal changes in the right and left ventricular contractions will not be the same in different experiments.

Tabulations of data indicate that this is the case. In all, thirty-nine experiments were analyzed, twenty-seven dealing with total systole and twelve with ejection phases. The following resultant effects may be tabulated:

	LEFT VENTRICULAR SYSTOLE OR EJECTION	RIGHT VENTRICULAR SYSTOLE OR EJECTION
Abbreviated	12	21
Unchanged	7	7
Lengthened	20	11
Total	39	39

When the results were further analyzed it was found that corresponding directional changes in right and left ventricular systole (or systolic ejection) occurred in eighteen experiments although many of these showed quantitative differences. In twenty-one experiments the durations of right and left ventricular systole changed in opposite directions or a change occurred only on one side. The detailed changes may be tabulated as follows:

	Number of cases
Systole increased absolutely in both ventricles	8
Systole decreased absolutely in both ventricles	10
Systole practically unchanged in left, increased in right	0
Systole practically unchanged in left, decreased in right	5
Systole practically unchanged in right, increased in left	4
Systole practically unchanged in right, decreased in left	1
Systole increased in left, decreased in right	8
Systole increased in right, decreased in left	3
	39

The changes in opposite direction were often marked. In one experiment for instance the left ejection increased 0.013 of a second while the

right ejection decreased 0.019 of a second. In another the left ejection decreased 0.018 of a second whereas the right increased 0.032 of a second.

While the many dynamic complications unfortunately made it quite impossible to untangle the factors determining variations in the durations of right and left ventricular systole, these observations are valuable in a general way for they illustrate how variable the durations of systole in the right and left ventricles may be when circulatory conditions are altered.

On analyzing these results as regards differences in the onset of contraction it was found that the differences were not very striking, as a rule. The onset of right ventricular systole was relatively delayed during compression in six cases, remained unchanged in nineteen and began relatively earlier as compared with the left ventricle in fourteen cases. As the difference in the first six cases mentioned was never more than 0.01 of a second it may be said that the onset of right ventricular contraction was affected in only fourteen cases, and in these the greatest change recorded was to advance right ventricular systole by 0.035 of a second. This operates so that when there is a right precedence in the control beats the asynchronism is increased and when the left side precedes in the control beats the asynchronism is lessened. In several instances the relation of the two ventricles was reversed, a left precedence before changing to a right precedence after compression. This relatively earlier onset of right ventricular contraction is probably referable to changes in conduction rates in the two ventricles resulting from the improved or altered coronary blood supply which accompanies the rise in aortic pressure.

An analysis of tabulated data from thirty-nine experiments showed that the differences in the termination of systole and systolic ejection were more striking. In the majority of experiments, i.e., in twenty-three, systole of the right ventricle terminated relatively earlier than in the control, the greatest change being 0.036 of a second. This means that the two ventricles end their contractions more nearly at the same time when the right ventricle continues to contract longer than the left in the control, but means a greater asynchronism when there is a right-sided precedence at the start. In a few cases the relative times of termination were actually reversed. In six experiments, however, the right ventricular contraction stopped relatively later, the maximum relative retardation recorded being 0.016 of a second. In ten experiments no relative change occurred.

An examination of graphic plots of these experiments showed that the differences in the asynchronism at the end were not dependent on the variations at the onset. In only 15 experiments were they in the same direction. In the others the change was either in opposite directions or

a change of one was associated with no change in the other. Even when the variations in onset and end were in the same direction they were quantitatively different, the alteration of the termination in such cases as a rule being the more marked.

Such differences in the cessation of right and left ventricular systole as are found in these experiments can be explained on the assumption that the dynamic factors controlling the duration of the contraction process (e.g., initial length and tension, arterial resistance) affect the two ventricles in unequal degree, and that irregular results arise owing to the dominance of one or the other of these factors. These experiments fail, however, clearly to separate the influences exerted by the different factors concerned.

C. Compression of pulmonary artery and marked lung inflation. With the hope of establishing more clearly the relative rôles played by changes in initial tension and aortic resistance, experiments were instituted in which the two ventricles were compelled to operate under opposite dynamic conditions. This was accomplished by increasing the resistance between right and left ventricles, viz., *a*, by compression of the pulmonary artery, and *b*, by extreme lung inflation. When the pulmonary artery was partially compressed just before its bifurcation the resistance against which the right heart ejects blood was augmented and the initial tension and volume increased. On the other hand, the aortic resistance diminished and the initial tension and volume of the left ventricle were reduced. The effects of these procedures were shown on the optically recorded pressure curves. As anticipated, the amplitude of the right ventricular and pulmonary arterial pressure curves increased, while the height of the left ventricular and aortic curves decreased. Accompanying these changes there was a rise in the initial tension of the right ventricle and in the diastolic pressure of the pulmonary artery, and at the same time a fall in the initial tension of the left ventricle and of diastolic pressure of the aorta. Similar though less marked changes accompanied extreme inflation of the lungs.

A careful study of tabulated results from twenty-eight experiments showed the following effects on the duration of right and left systoles.

	LEFT VENTRICULAR SYSTOLE OR EJECTION	RIGHT VENTRICULAR SYSTOLE OR EJECTION
Abbreviated	7	14
Unchanged	8	2
Lengthened	13	12
Total	28	28

When the results were further analyzed it was found that corresponding directional changes occurred in 14 cases although many of these showed quantitative differences. In the other fourteen experiments, the duration of right and left ventricular systole changed in opposite direction or a change occurred only in one side.

These effects may be tabulated as follows:

	Number of cases
Systole increased absolutely in both ventricles	10
Systole decreased absolutely in both ventricles	4
Systole unchanged in left increased in right	3
Systole unchanged in left decreased in right	5
Systole unchanged in right increased in left	0
Systole unchanged in right decreased in left	2
Systole increased in left decreased in right	3
Systole increased in right decreased in left	1
	28

As regards the onset of contraction no relative changes were found in 9 experiments. In 15 experiments the onset of right ventricular systole occurred relatively earlier than that of the left, the greatest change being 0.015 of a second. In four the reverse was the case, the change being less than 0.01 of a second.

The relative changes in the termination of right and left ventricular systole followed the relative changes in onset in thirteen experiments, but in the remainder the changes were either in opposite directions, the relation of the onsets was unchanged or that of the terminations was unchanged.

In 16 out of 28 experiments contraction terminated relatively earlier on the right side, the greatest change observed being 0.038 of a second. In 8 experiments, however, right ventricular systole terminated relatively later after compression or lung inflation, the greatest change being 0.019 of a second. In four, no change was observed.

While these experiments again demonstrated the ability of the two experiments to vary both their onset and end in an independent manner, it is obviously impossible to draw clear conclusions as to the specific factors determining the changes noted.

D. The effects of asphyxia. Previous experiments reported by Katz and Wiggers (2), (5) have shown that when the vagi nerves are cut, the initial effects accompanying cessation of artificial respiration consist in a marked abbreviation of left ventricular systole. The possible factors concerned have also been analyzed. In the present investigation the comparative effects on the right and left ventricles were studied in eight experiments. It was found that the durations of systole decreased to a marked degree in both ventricles. The abbreviation was unequal, how-

ever, in the two ventricles, the right side being more affected in five experiments and the left in three. In one experiment, no abbreviation occurred in the right ejection phase although the left showed a marked tendency to become shorter.

Changes in the onset of systole were slight and in only one instance exceeded 0.018 of a second. In five experiments right systole and ejection began a little earlier, in two a little later, while in the remaining two no variations could be established. Again variations in the right and left ventricular systoles were largely due to differences in the termination of contraction. The nature of these differences is indicated in figure 8. It will be seen that the relative onsets of contraction do not change appreciably and that the duration of the left ventricular systole is obviously less while that of the right ventricle actually increases slightly toward the end (*E* and *F*). Such unequal changes in the length of systolic ejection obviously cause variations in their relative terminations, as is clearly shown. Such changes occurred in about half of the experiments. In the other half the effects were exactly opposite, however; the right ventricular contraction terminated earlier than the left.

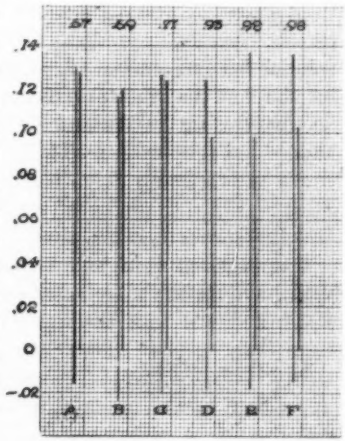

Fig. 8. Plot showing the effect of asphyxia on the asynchronism of right and left ejection and their durations. Constructed as figure 6. In each couple of lines, the left hand one indicates right ejection and the right hand one, left ejection. Sequence of events obtained by starting at the bottom and reading upwards. Figures on top indicate duration of cycle in seconds. *A* is the control; *B*, *F*, various stages of asphyxia. Ordinate figures in seconds. Discussed in text.

Significance of results. With these facts established we are in a position to point out several facts regarding their significance.

In the first place, these observations are of clinical interest for they give a hint that in such conditions as aortic stenosis, aortic insufficiency and marked arterial hypertension the two ventricles may not be acting synchronously but that such variable relations of right and left-sided contractions as are found on aortic compression in animals may readily occur. Again, the circulatory effects produced by mitral stenosis, emphysema, congenital pulmonary stenosis, etc., may possibly produce such types of asynchronism as were found on compression of the pulmonary artery and extreme lung inflations. They offer in addition a

simple rational explanation for the occurrence of several clinical signs, among which may be mentioned *a*, the split second heart sound which is heard fairly frequently, and the occasional occurrence of such a splitting during one portion of the respiratory cycle only; *b*, the split R wave often seen in the normal electrocardiogram.

In the second place, they clarify certain dubious changes observed in accurately recorded volume curves of the ventricles. Thus it is possible that an asynchronous ejection of the two ventricles may account for certain changes in gradient observed in the systolic portion of such ventricular volume curves (2) while the asynchronous ending may account for the fact that the terminations of systolic ejection as determined from aortic pressure curves and volume curves do not always agree exactly. Indeed, as the two sides of the heart are asynchronous and the volume curve is simply an algebraic bi-plethysmogram, work diagrams such as Straub (14) and others have constructed from volume and left intraventricular curves become almost valueless.

But perhaps more significant than all is the bearing these findings may have on the interpretation of the T-wave of the electrocardiogram. Having shown that the right ventricle as a rule contracts for a longer time than the left under normal experimental conditions, and knowing that an active muscle is electro-negative to an inactive one, we can clearly appreciate how an electrical potential difference toward the end of systole can produce a monophasic deflection. If these assumptions are at all correct we can appreciate why, as Bartos and Burstein (15) have shown, the duration of the electrical systole as determined from Q to end of T, varies independently of mechanical systole determined from the heart sounds.

Finally, the variations in the relative terminations of right and left ventricular systole produced by many changes in the circulation may account for alterations not only in the amplitude but in the direction of the T wave. Daly (16) has already pointed out that the height of the T wave changes in animals following aortic and pulmonary compression, the results being quite variable. He, however, has given a different and, in my opinion, a less reasonable interpretation. More recently, Bartos and Burstein (15) have reported complete inversion of the T wave when stimulation of the central end of the vagus causes a pressor reaction of blood pressure.

It is obvious from these suggestive experiments that the relation of the T wave to the asynchronous ending of right and left ventricular contractions merits further careful investigation.

SUMMARY AND CONCLUSIONS

It has generally been tacitly assumed that the two ventricles initiate and terminate their contractions simultaneously. In order to reinvestigate this question the right and left intraventricular pressures or the aortic and pulmonary arterial pressures were recorded simultaneously and without any parallax. From the former curves, the relative onsets of the isometric contractions and ends of systole were calculated; from the latter, the moments of ejection and the end of systole were determined. The principal observations and deductions drawn from this study are as follows:

1. During normal experimental conditions, right and left ventricular systoles and also their ejection phases neither begin nor end simultaneously and as a rule are of unequal duration. In twenty-four experiments, right ventricular systole or ejection preceded in eleven instances by 0.016 to 0.027 of a second; whereas in an equal number of cases there was a left ventricular precedence of 0.013 to 0.03 of a second. In only two cases, contractions began simultaneously in the two ventricles. Right ventricular systole outlasted that of the left by 0.015 to 0.032 of a second in twenty cases, terminated earlier in three cases and ended synchronously in one. Apparently the time of termination bears no relation to the onset. Consequently, the duration of the contraction process varies independently in the two ventricles. Systole or systolic ejection was longer in the right than in the left ventricle in fifteen experiments, equal in four and shorter in five. However, a comparison of the ejection phases alone consistently showed longer periods on the right side. This indicates that the mean velocity of ejection must be slower on the right side.

2. The phasic relations of the contractions in the two ventricles are readily altered by vagal influences. Stimulation of the peripheral end of the vagus caused at first erratic and often reversed relations in the onset of right and left ventricular systole; on the whole, the tendency was to advance the relative onset of right ventricular contraction as compared to the left. All of these effects are suggestive of modifications of impulse conduction below the common A-V bundle. In the majority of experiments it tended also to prolong right ventricular contraction more than left ventricular contraction, the net result being that in the majority of experiments the ventricular systole or its ejection phase was longer in the right ventricle than in the left.

Upon recovery from vagal stimulation both ventricular systoles remained proportionately long even when a normal or slightly increased heart rate had been reëstablished. This prolongation persisted longer in the left ventricle, however, so that the ratio of systole length was in favor of the left ventricle. These changes occurred quite independently of

minor changes in the relative onsets, during recovery. These opposite effects and after-effects of vagal stimulation indicate that they are not due to direct influences of the vagus nerve on the ventricle; but are more probably secondary to dynamic changes produced.

3. Experiments designed to study the primary influence that differing initial tensions or variable arterial resistances might have on the asynchronism of the right and left ventricles proved to have been too complex. Many variations in the phasic relations of the two ventricular contractions resulted. Thus infusion of saline caused a lengthening of both ventricular systoles, but sometimes the effects were more marked on the right, at other times on the left side. Compression of the aortic and pulmonary artery caused a variety of changes in the durations and relative onsets and terminations of the two systoles. The abbreviation of systole produced by asphyxia was unequal on the two sides and followed no constant rule.

While it was therefore not possible to determine the precise cause of the variables effect in many instances the changes as a whole indicate that unequal initial tension and arterial resistance changes in the two ventricles play an important rôle in determining the duration of the contraction process in the right and left ventricles. In addition, however, other factors as yet not understood, must be taken into consideration.

4. It follows from these findings that changes in one ventricle do not necessarily indicate the changes in the other. The two ventricles must be considered separately, and the presence of an asynchronism at the onset and end of right and left ventricular contraction borne in mind when fine analysis of cardio-vascular phenomena are made. In many instances these observations may be ignored, in others a disregard of these factors may lead to erroneous deductions.

5. Finally, the demonstration that right and left ventricular contractions do not begin and end simultaneously, suggests a simple and reasonable explanation for some cardio-vascular phenomena which heretofore were more or less baffling. Probably the most significant is the explanation of the T wave in the electrocardiogram by the asynchronous cessation of right and left ventricular contraction.

In conclusion, I wish to express my gratitude to Dr. C. J. Wiggers, at whose suggestion this investigation was started, for his advice and criticisms. I also wish to acknowledge the assistance I received from Messrs. Boettner, Baker, Dvorak and Weinman in performing some of these experiments, and in the case of the first mentioned for his help in tabulating some of the data.

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